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THE RING ROT BACTERIUM IN RELATION TO TOMATO AND EGGPLANT [1]

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INTRODUCTION

Bacterial ring rot, incited by *Corynebacterium sepedonicum* (Spieck. & Kotth.) Skapt. and Burkh. is the most serious of recently introduced diseases of the potato (*Solanum tuberosum* L.). It has been reported as occurring in nature only on the potato. Spieckermann and Kotthoff [2] reported negative results in artificial inoculations of aboveground stems of potted plants of tomato (*Lycopersicon esculentum* Mill.) and related species, *L. humboldtii* (Willd.) Dunal, and *L. pimpinellifolium* Mill. (*L. racemigerum* Lange) in the field during 1910 and 1911. However, in 1913, in additional inoculations to 19 field-potted plants each of *L. esculentum* and *L. racemigerum*, 9 "diseased plants" in each of the 2 lots were recorded. No mention was made, however, of plant symptoms or of reisolation of the organism. *Solanum commersonii* Dunal and *S. citrullifolium* A. Br. were also reported as "susceptible."

Using tomato as a test plant, Stapp [3] observed occasional local withering of the leaves of young transplants a short distance above and below the point of stem inoculation. Reisolations were made after 3 to 4 months, at distances of 20 and 25 cm. below the site of the inoculation. Fruit infection was not reported and the organism was regarded as being "mildly pathogenic." In inoculation tests on field pea (*Pisum arvense* L.) and bean (*Phaseolus vulgaris* L.), the ring rot organism proved to be "slightly pathogenic." *Corynebacteruim sepedonicum* was reisolated from the three hosts infected. More recently Savile and Racicot [4] reported that the pathogenicity of the bacterial ring rot organism in young artificially inoculated tomato plants was markedly greater than that described by Stapp. They pointed out that symptoms of leaf wilting were evident in 19 days, at which time the tissues were quite heavily invaded by the bacteria. All tomato plants inoculated were severely affected, extensive yellow-walled stem cavities being formed, after which the plants soon died.

During early studies on the host range of the ring rot organism in the greenhouse at Madison, Wis., it was found that young tomato transplants (variety, Bonny Best) were very easily infected. Artifi-

[1] Received for publication May 21, 1943. The photographs that accompany this paper were made by Eugene H. Herrling.

[2] SPIECKERMANN, A., and KOTTHOFF, P. UNTERSUCHUNGEN ÜBER DIE KARTOFFELPFLANZE UND IHRE KRANKHEITN. 1. DIE BAKTERIENRINGFÄULE DER KARTOFFELPFLANZE. Landw. Jahrb. 46: [659]–732 illus. 1914.

[3] STAPP, C. BEITRÄGE ZUR KENNTNIS DES BACTERIUM SEPEDONICUM SPIECKERM. ET KOTTH., DES ERREGERS DER "BAKTERIENRINGFÄULE" DER KARTOFFEL. Ztschr. f. Parasitenk. 2: [756]–823, illus. 1930.

[4] SAVILE, D. B. O., and RACICOT, H. N. BACTERIAL WILT AND ROT OF POTATOES. Sci. Agr. 17: 518–522, illus. 1937.

cially inoculated test plants on being held in a warm greenhouse (24° C.) for a short period developed conspicuous wilting and withering, followed by unilateral chlorotic bleaching of the upper leaflets. A preliminary report [5] on bacterial ring rot in relation to the tomato has been published. The present paper is a report of investigations on the relation of the ring rot organism to the host tissues of tomato, on the disease development and resistance in tomato and eggplant, and on host range studies.

MATERIALS AND METHODS

Tomato plants (variety Bonny Best) used in the histological phase of this study were grown both in the greenhouse and in the field. The culture of the organism was a single-colony isolate from a fourth serial dilution plate of a laboratory stock culture isolated originally from an affected tuber collected in north-central Wisconsin and held in storage at 5° C. in inoculated Irish Cobbler potato tubers. The inoculum used in all cases was from 7-day-old pure culture on freshly prepared slants of potato dextrose agar containing neutral yeast extract grown in the dark at 20° to 22° C. Inoculations were made either by dipping a needle point into the culture and inserting it directly into the vascular region or by puncturing with a needle through a water suspension of the organism into the vascular tissue of the lower stem. Both types of inoculation were very successful. All greenhouse-inoculated test plants were held at an air temperature of about 24° C.

The plant tissues selected for histological study were immediately killed and fixed by placing sections directly in a solution consisting of 10 cc. of commercial 40-percent formalin, 5 cc. of glacial acetic acid, and 100 cc. of 50-percent ethyl alcohol. All tissues were evacuated under light suction while in the fixative. The fixed material was dehydrated following the tertiary-butyl-alcohol schedule and embedded in tissue-mat paraffin with a melting point at 52° to 54° C. according to the usual procedure. Transections and longisections were cut 8 to 10 microns in thickness and mounted serially to facilitate more complete observations on the path of vascular invasion by the bacteria. Miscroscopical examinations of histological material were in most cases paralleled by isolations from similar portions of the host tissues.

Reed's [6] Gram-stain formula and schedule were followed for the bacteria, which are strongly Gram-positive. The host tissues were then differentiated by counterstaining with safranine (1 percent in 95-percent ethyl alcohol) followed by fast green (saturated solution in absolute ethyl alcohol). This combination was found to be most satisfactory. In sections stained by this method the vessel walls retained a brilliant red, while the walls of the parenchymatous cells were differentiated by a deep green. This counterstaining in no way modified the preliminary deep-blue Gram stain of the bacteria in the host tissues.

[5] Larson, R. H., Walker, J. C., and Fogelberg, S. O. Bacterial ring rot in relation to the tomato. (Abstract) Phytopathology 31: 14–15. 1941.

[6] See Racicot, H. N., Savile, D. B. O., and Conners, I. L. Bacterial wilt and rot of potatoes—some suggestions for its detection, verification, and control. Amer. Potato Jour. 15: 213–318, illus. 1938.

EXPERIMENTAL RESULTS

INOCULATION AND ISOLATION

The bacterial organism was consistently recovered from the vascular system of stem and petiole of tomato plants artificially inoculated with the organisms. Transfers from resulting isolations were checked by needle inoculations to stems of known healthy potato test plants (varieties Green Mountain, Katahdin, and Triumph) on which typical systemic vine and tuber symptoms of bacterial ring rot were readily produced. In all cases the isolates from infected tomato plants exhibited in the potato the same degree of virulence as did the original culture.

SYMPTOMS

Symptoms in tomato plants systemically infected with the bacterial ring rot organism were of two distinct phases consisting of an early

FIGURE 1.—Systemic infection of young Bonny Best tomato plant, growing in the greenhouse at 24° C., 4 weeks after inoculation of the lower stem with the bacterial ring rot organism. Irregular drooping and wilting of the upper leaves, necrosis of lower leaves, and slight petiole epinasty are evident.

wilting and withering stage and a necrotic to moribund stage. The earliest symptoms of systemic invasion were evident within 8 to 12 days after inoculation, as a dull or dark-green coloration and slight flaccidity of the young apical leaflets. Concurrently a gradual irregular wilting, loss of firmness and drooping of the lower leaves was

evident, accompanied by a slight upward and inward marginal rolling of the lower leaves and subsequent slight downward turning of the petioles (fig. 1). Infected young tomato transplants, however, did not show a marked stimulation response of petiole epinasty preceding or following leaf wilting or flaccidity. On subsequent days as systemic invasion progressed irregular mottling approaching chlorosis appeared in the lower leaves as the color of the entire plant partly or wholly changed to a pale green with bleaching of the interveinal areas. Infected plants were more or less stunted. Infection commonly progressed on one side of the stem to adjacent petioles, rachis, and petiolule, causing a conspicuous retarded leaflet expansion and unilateral leaf development (fig. 2, *A*). Following these symptoms, the invaded plants took on a yellow cast with marginal leaf rolling and interveinal bleaching or browning. In addition, slight irregular necrotic streaking commonly occurred on the stems. This type of stem streaking was often followed by a conspicuous exudation of creamy-white or yellowish, slimy bacterial masses at the points of tissue break-down in the nodal region where the bacteria had spread outward through the cortical stem tissue (fig. 2, *B*). There was not a strong tendency, however, for the bacteria to come to the surface through fissuers in the stem or petiole tissues. When stems of infected plants were cut lengthwise, slight disintegration of the pitch cylinder in the form of small creamy-white to yellowish cavities were visible. Transections of the petiole and rachis, on the other hand, showed only a very slight water-soaked area adjacent to the vascular elements, in which could be seen a creamy-white bacterial ooze when slight pressure was applied.

Older plants showed noticeable stunting with shortened and somewhat thickened internodes. In cases of severe systemic infection an irregular wilting occurred in the upper portion of the plants when the only sign of infection was a slight chlorotic, shriveled condition of the lower leaves. Infected plants in the field which did not succumb early in the season showed the most pronounced symptoms (fig. 3). Pedicel and flower drop in infected plants in the field were not very common.

Distortion of infected fruit was not observed. However, internal fruit infection was manifest by the outward appearance of somewhat shriveled, soft, irregular, water-oaked areas at or near the torus end (fig. 4, *A*). In sectioned mature fruit internal macroscopic symptoms were evident as a more or less creamy liquefied disorganization of the affected areas. The creamy-white strands of bacterial masses were traced through the placental region and locular cavity directly to the fleshy covering of the seeds. Size of seed was much reduced and the number of aborted and immature seeds was greatly increased in many cases (fig. 4, *B*). In ripe severely infected fruit the entire inner pulp might be completely liquefied but held intact by the shriveled skin.

RELATION OF THE BACTERIA TO THE HOST TISSUE

The movement and distribution of the ring rot organism in the tissues of the tomato plant and fruit were ascertained primarily by microscopic examination of stained serial sections, and in most cases paralleled by stained smears and isolations from similar portions of the host. Systemic invasion of the stem tissues of the tomato occurred

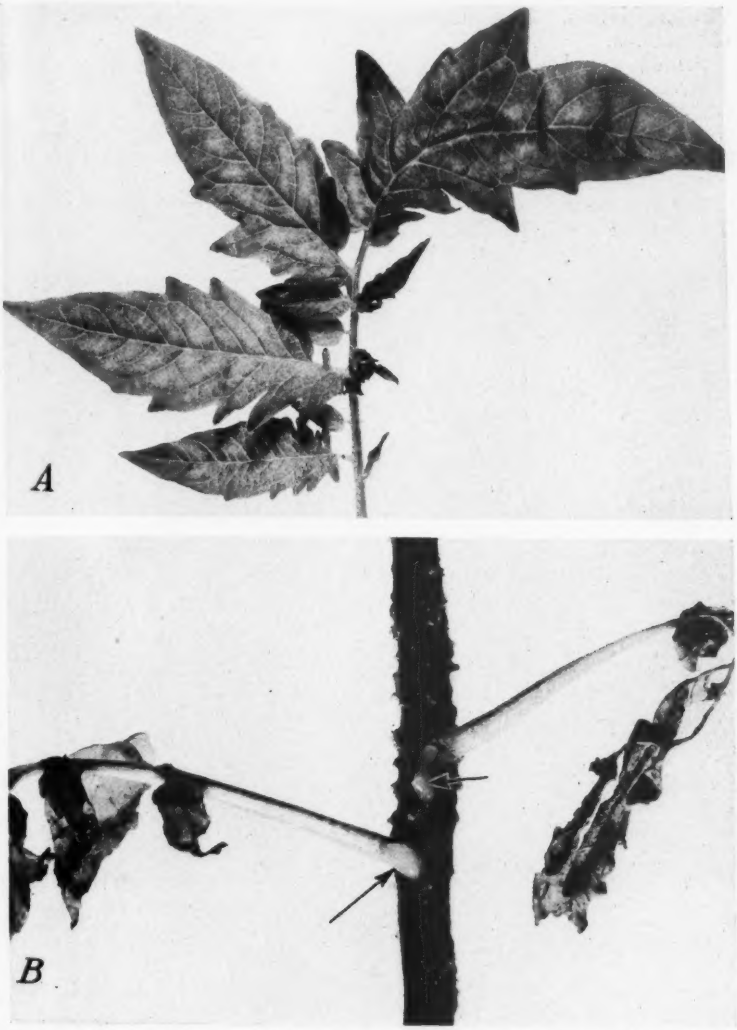


FIGURE 2.—*A*, Unliateral leaf development resulting from systemic invasion by the ring rot organism of one side of petiole, rachis, and midrib of an upper leaf of a young tomato plant. *B*, Exudation of wet, glistening, creamy-white, sticky bacterial masses near leaf nodes following break-down of cortical tissues of a severely infected tomato stem.

largely in the vascular spiral and annular vessels of the xylem elements; the adjacent pitted vessels were less frequently invaded (pl. 1, *A*, *B*). Immature xylem elements and cells of the cambium were only occasionally infected. The outer and inner phloem elements were rarely invaded by the bacteria except in complete break-down of the

vascular tissues. Bacteria were not observed in the ray tissue of the older stems.

Seldom were bacteria found in the parenchymatous tissues adjacent to invaded xylem except following tissue rupture near the point of inoculation or at points in the invaded stem where xylem vessels became thickly populated with bacteria and enzymic dissolution of the thinner, weaker-walled xylem cells took place. In these cases small lysigenous cavities in the parenchymatous tissues were formed which were filled with bacteria, and thickened cell walls could be seen lying free within the cavities. In the dissolution of the nonlignified cell walls the bac-

FIGURE 3.—Bonny Best tomato plant, growing in the field, systemically infected with the ring rot organism, showing stunting, irregular wilting, leaf chlorosis and very light fruit set.

teria progressed only slightly from the parenchyma cavities into the surrounding intercellular spaces.

Cells of the xylem elements in systemically infected stem tissues did not show disintegration, break-down, or increase in lignification. The transformation of adjacent parenchyma tissues into sclereids was not seen. However, thinner, weaker xylem walls were found in many cases, indicating enzymatic action in advance of the bacteria. This solvent action was also seen in many cases in advance of the bacteria in the intercellular spaces and out from the small lysigenous cavities in the parenchymatous tissues.

In the tissues of the petiole, rachis, and stalk of the leaf, the progressive movement of the ring rot bacteria into the xylem elements was

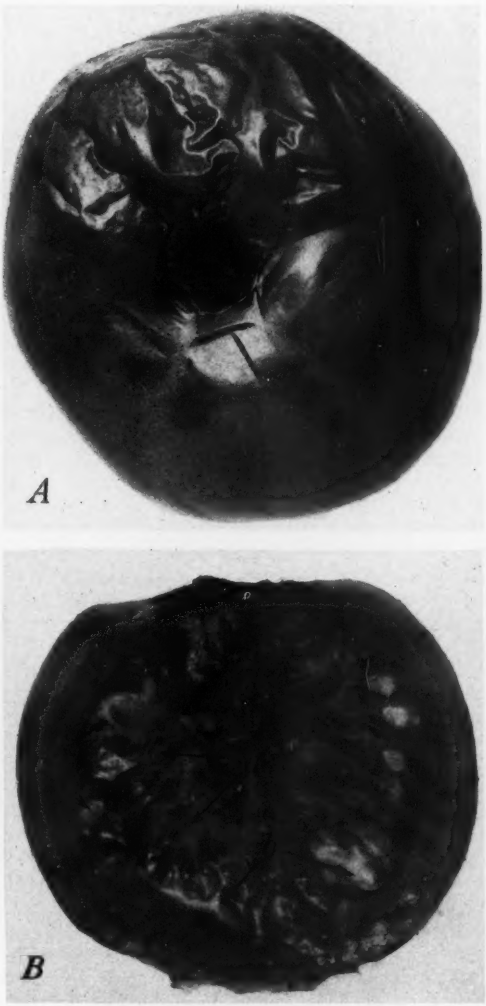


FIGURE 4.—Systemically infected ripe tomato fruits from a diseased plant. *A*, External symptoms on the fruit appearing as irregular, soft, water-soaked areas near the torus end. *B*, Longisection of fruit showing (*a*) bacterial masses in the vascular elements of the placental region appearing as creamy-white strands; (*b*) bacterial aggregates in disintegrated tissues in the locular cavity; (*c*) aborted seeds.

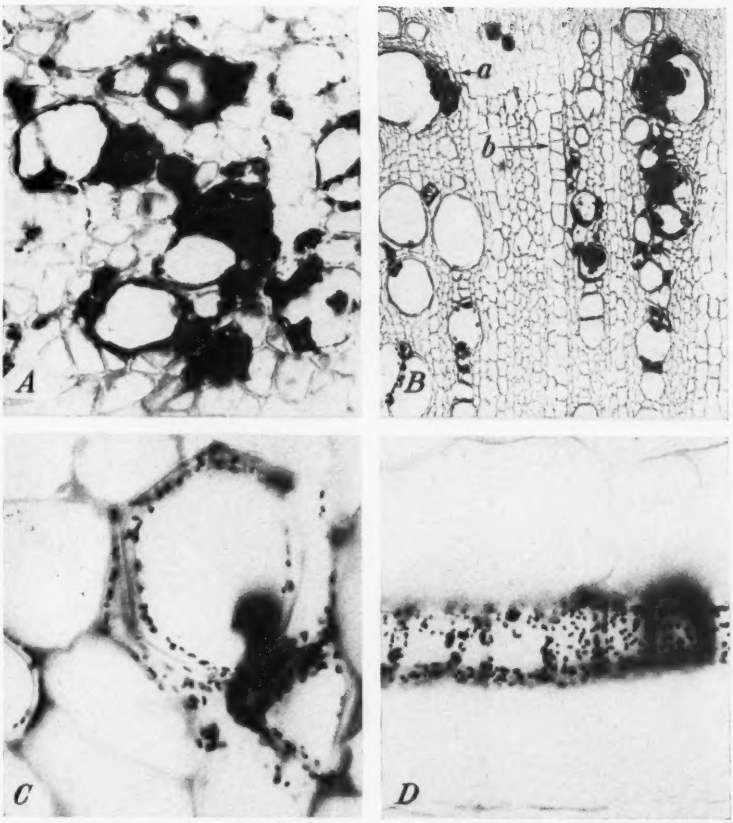
traced from the infected vascular elements of the stem, which in turn gave rise in the petiole to the median or lateral traces. In some cases, however, the vascular tissue of the petiole was invaded via the intercellular spaces of the parenchyma when the same tissues in the stem were similarly affected. Infection of the petiole and rachis was, as in the tissues of the stem, restricted to the spiral and annular elements of the xylem vessels (pl. 1, *C*). Transections of invaded petiolule tissues showed the ring rot bacteria present in the xylem vessels (pl. 1, *D*), and to a slight extent in the spaces between the small parenchyma cells about the heavily infected elements. Cells of the endodermallike tissue, in addition to the xylem elements of infected petioles, were also invaded (pl. 2, *A*) while adjacent cortical and pericycle cells remained free of bacteria. In the latter region, the bacteria were present in relatively smaller numbers than in the corresponding tissue of the petiole. In transections of infected leaflets the organism was seen to be confined entirely to the xylem vessels, many of which were completely filled with bacteria (pl. 2, *B*). In the areas of the leaf blade where the spiral elements of the lateral and branch veins were heavily infested, the cells of the spongy parenchyma were seen to have large numbers of bacteria adhering to their outer walls.

It was found by numerous stained smears, repeated isolations, and the examination of serial histological sections that the systemic advance of the organism in the underground adventitious root tissues of infected tomato plants was somewhat less rapid than in stem and petiole; the difference, however, was not great. The movement of the bacteria in the root tissues was restricted as in the stem to the xylem vessels (pl. 1, *A*). In advanced stages of root infection the cambium became infected to a slight extent and small cavities in the contiguous parenchyma were also to be found. The spread of the organism into the root cortex was not observed and no sclereids were found in any tissues of the root invaded by the ring rot organism.

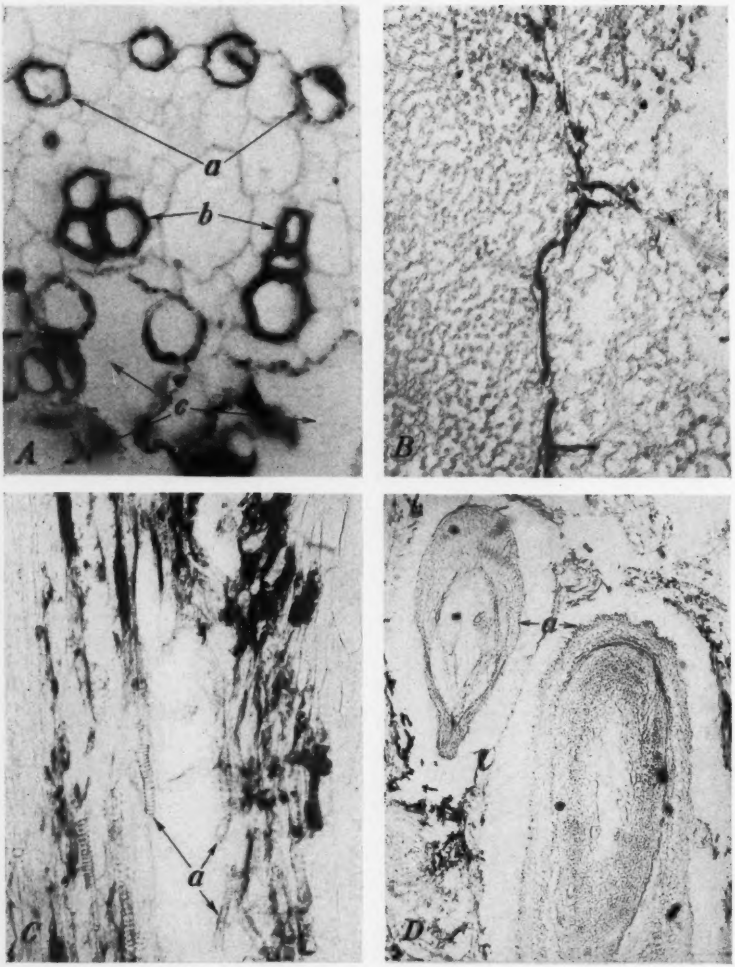
Systemic infection of fruit spurs and fruit of infected plants was first ascertained by stained smears and isolations from portions of the base of the peduncle, the pedicel, the torus, internal fruit tissues, and seed. On examination of histological sections of the different fruit-spur tissues, the ring rot organism was found to be confined entirely to the thick-walled xylem elements. As in the petiole and rachis, unilateral infection of the xylem traces was observed and the path of infection was traced from the traces in the fruit spur to the xylem elements of the stem.

In stained sections of portions of infected mature fruit the ring rot bacteria were traced from the place of fruit attachment through the spirally thickened tracheae of the fleshy placental region (pl. 2, *C*) and locular cavity, directly to the seeds. In the locular cavity large numbers of aborted seeds surrounded by bacterial masse swere commonly found (pl. 2, *D*). In such cases the cells of the integument of the seed coat do not mature.

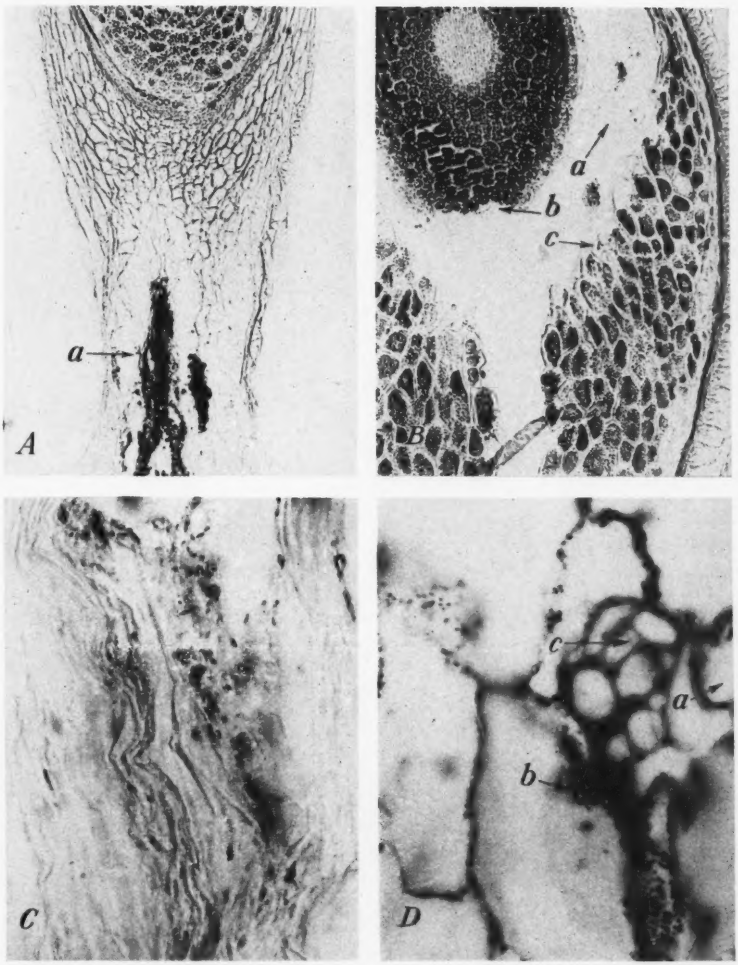
When serial transections and longisections of seeds from systemically infected fruit were examined, bacterial masses were found to be abundant outside the spiny layer of the seed coat and among the hairs formed by the epidermal layer of the seed coat. Large numbers of bacteria were also observed within the spiral vessels in the hilum region and in the funicular tissues remaining attached to the seed



A, Ring rot bacteria in secondary xylem vessels of tomato root. Transection, X 504. *B*, Cross section of portion of stem of mature tomato plant 60 days after inoculation; secondary mature pitted xylem next to cambium (*a*) and ray (*b*) tissues showing bacteria only in the large xylem elements. Transection, X 165. *C*, Systemically infected xylem vessels of the petiole with bacteria also in parenchyma tissue as a result of movement through disintegrated tracheal walls. Transection X 1429. *D*, Spiral vessels of petiolule with abundant bacteria. Transection, X 1142.



A, Transection of infected portion of a tomato petiole showing masses of bacteria in endodermallike cells (*a*) and xylem elements (*b*) and large lysigenous cavities in adjacent parenchmatous tissue (*c*). X 504. *B*, Portion of longisection of lamina of leaf with abundant bacteria in xylem spiral elements of small branch veins. X 160. *C*, Longisection of systemically infected mature tomato fruit showing spiral thickenings of the tracheae (*a*) of the fleshy placental region lying free amid cell debris and bacterial masses; such infected bundles appear macroscopically as cream-colored streaks in severely infected fruit. X 79. *D*, Aborted seeds (*a*) surrounded by bacterial masses in the placental region and lysigenous cavities, in mature infected fruit. Size of seeds is reduced and the number of aborted seeds greatly increased. X 79.



Longisections of tomato seeds (*A* and *B*) from systemically infected fruit and seedlings (*C* and *D*) grown from seed of infected fruit. *A*, Immature seed somewhat aborted, showing portion of the seed and funiculus of the seed; vascular elements of the funiculus including xylem elements and contiguous tissues are destroyed by the ring rot bacteria (*a*). X 125. *B*, Mature seed with bacteria within the seed in the micropylar region (*a*) between the embryo (*b*) and endosperm (*c*). X 125. *C*, Ring rot bacteria in metaxylem elements of hypocotyl tissue of a young seedling grown from infected seed. X 1142. *D*, Stem tissue of young plant grown from infected seed, showing bacteria in pith parenchyma (*a*) and intercellular spaces (*b*); the inner phloem cells (*c*) are not invaded. X 1070.

(pl. 3, *A*). In many cases the bacteria had invaded the parenchyma of the middle layer of the seed coat, and also the region between the integument and the endosperm. Invasion of the micropylar region of the seed between the embryo and endosperm was also observed (pl. 3, *B*).

SEED TRANSMISSION

Evidence has been presented in the histological studies that the bacterial ring rot organism will invade the seed of systematically infected tomato fruit and become established in the spiral vessels in the funiculus of the seed, in the micropylar region between embryo and endosperm within the seed, and on the seed in the roughened integument or seed coat. The viability of the bacteria on the seed coat and within the seed has been proved by culture methods and inoculation.

Seed transmission tests were made with seed harvested from mature field-grown systematically infected fruit. Plantings were made in flats in steamed soil and the seed covered with washed steamed sand. All seeds planted were treated for external contamination with 1–1,000 mercuric chloride, for 3 minutes and rinsed in several changes of sterile water. Plantings were made in the greenhouse at temperatures between 24° to 26° C., which are the most favorable for germination and emergence of tomato seed. The planted flats were watered in such a way as to avoid spattering or splashing the emerging seedlings. Check plantings with seed harvested from known healthy mature fruit from a separate field planting were included. The first plantings were made with seed stored at room temperature for a period of 1 month after harvest and the second plantings were made with seed held 6 months in storage. A total of 30 lots of seed were harvested from infected fruit for the seed transmission tests (table 1).

TABLE 1.—*Emergence of seed lots from infected and healthy fruit, 15 days after planting; 35 seeds in each planting*

Fruit No.	Emergence		Fruit No.	Emergence	
	First planting	Second planting		First planting	Second planting
	Number	*Number*		*Number*	*Number*
1	19	24	19	16	21
2	27	31	20	8	12
3	31	28	21	27	29
4	7	9	22	20	14
5	20	16	23	12	17
[1] 6	32	31	[1] 24	31	33
7	[2] 26	29	25	12	9
8	[2] 21	16	26	24	19
9	[2] 23	20	27	30	26
10	[2] 7	9	28	9	17
11	[2] 8	12	29	23	16
[1] 12	[2] 30	31	[1] 30	34	35
13	25	21	31	26	29
14	14	16	32	15	27
15	27	21	33	26	21
16	29	28	34	19	13
17	24	27	35	21	14
[1] 18	34	32	[1] 36	32	34

[1] Healthy fruit.
[2] Figure 5.

Two weeks after planting an examination of the seedling flats showed a marked difference in percentage of germinability and uniformity of growth of the seedlings in the respective lots (fig. 5). In the emergence of seed harvested from infected fruit the seed coat invariably was carried up above ground. This was very much less evident in seedlings growth from seed of healthy fruit. As the seedlings from seed of infected fruit emerged the curved hypocotyl tissues of the young seedlings were in direct contact with the hilum tissues of the seed coat, and in many cases after emergence the apex of the immature and deformed cotyledonary leaves was held together by the seed coat for some time (fig. 6, *B*, *C*, and *D*).

In addition to histological sections (pl. 3, *C* and *D*), numerous smears made from a large number of emerging seed coats, seedling

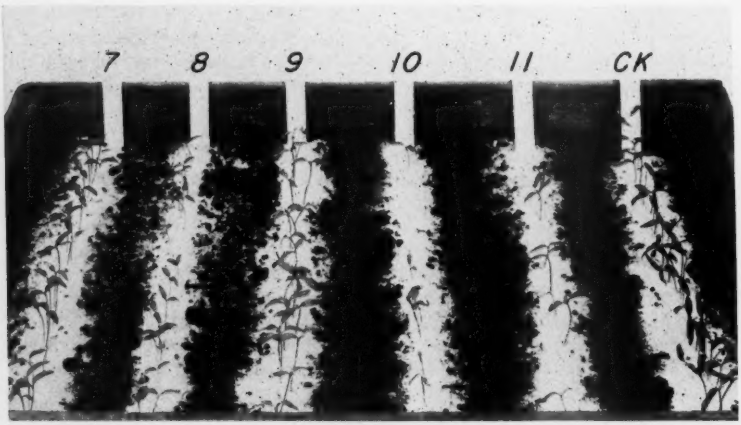


FIGURE 5.—Plantings of tomato seeds harvested from mature infected fruits (first planting Nos. 7 to 11 in table 1) showing various percentages of nonemergence. The last row on the right was planted with seed from healthy mature fruit.

cotyledons, hypocotyl and stems tissues and stained with Gram's stain showed the ring rot organism to be present in great numbers. Isolations from infected seedling material were identified by the Gram's stain test as well as by inoculations to young Triumph potatoes and to tomato seedlings.

TRANSMISSION THROUGH INOCULATED SOIL

All soil inoculation tests were conducted in an aerated mixture of 3 parts of greenhouse composted soil to 1 part of sand autoclaved in 8-inch clay pots. A portion of the prepared soil was inoculated by thoroughly incorporating a water suspension (100 cc.) of 7-day-old pure cultures of *Corynebacterium sepedonicum* into each 8-inch pot used. Young, healthy tomato transplants were set directly in the freshly inoculated soil. In two series of 40 pots each held in the greenhouse at about 24° C., all plants showed distinct apical dark-green coloration and flaccidity in about 9 days after transplanting. This condition was followed by typical stunting of the plants with shriveling, chlorosis, and necrosis of the lower leaves and unilateral

wilting of the apical leaves. All control plants in uninoculated soil to which only sterile water was added, remained healthy.

Histological examination of longisections of numerous tomato roots removed after being exposed to the inoculated soil for 5 days showed very definitely that the avenue of root infection was greatest by far through the wounded root tissues; few cases were observed in which infection took place through what appeared to be uninjured root cortex.

Inoculated soil held in the greenhouse at 20° to 22° C. in a moist condition in flats for 8 weeks and planted to young tomato plants gave

FIGURE 6.—Systemic invasion of tomato seedlings from infected seed. *A*, Seedling from noninfected seed. *B*, *C*, *D*, seedlings grown from seed collected from mature systemically infected fruit. Stunted and distorted cotyledonary and primary leaves are to be noted and also the adherence of the seed coat to the cotyledons.

only slight infection, 7 affected plants appearing out of 437; no infection occurred when inoculated soil was planted after being held for 12 weeks. When seeds were planted in inoculated soil that had been held in 12-inch pots over winter in the filed, no ring rot infection resulted.

When inoculum (300 cc. water suspension) was applied to the soil surface around healthy young undisturbed tomato seedlings of transplanting age growing in flats, infection rarely resulted. Out of 720 seedlings treated in this manner, only 6 became infected; the bacterial ring rot organism was recovered from those seedlings.

These results indicate that although infection through the wounded root system of tomatoes occurs readily in inoculated soils, the organism does not persist long in the soil. However, when bacterial ring rot has occurred in a field planting of potatoes, the immediate replanting of this ground to tomatoes is not advisable. Moreover, the possible transmission of the disease to young tomato transplants from ring rot infected potato tubers and volunteer potato plants is obvious.

REACTION OF TOMATO VARIETIES TO CORYNEBACTERIUM SEPEDONICUM

To ascertain symptom differences between tomato varieties artificially inoculated with a pure culture of the bacterial ring rot organism, tests involving 70 plants each of 22 commercial varieties and the currant tomato (*L. pimpinellifolium*) were conducted in the greenhouse. Data on severity of symptoms were taken at two phases: (1) at 10 days after inoculation, in the early wilting and dull-green apical leaflet coloration phase, and (2) at 30 days, as the necrotic to moribund phase became pronounced. As recorded in table 2, the

TABLE 2.—*Severity of symptoms in tomato varieties artificially inoculated with Corynebacterium sepedonicum* [1]

Variety	Severity of infection after 10 days (early phase)	Severity of infection after 30 days (late phase)	Variety	Severity of infection after 10 days (early phase)	Severity of infection after 30 days (late phase)
Bonny Best	3	3	Morse's Special Early No. 498	3	1
Bounty	2	3	Pan America	3	3
Chicago	2	2	Pritchard	1	1
Currant	1	1	Pritchard Scarlet Topper	2	2
Del Monte	2	2	Riverside	1	1
Early Baltimore	2	2	Rutgers	2	2
Early Prolific	2	2	Stokesdale	2	3
Early Rutgers	3	3	Supreme Marglobe	3	3
John Baer	1	1	Texas Early	3	1
J. T. D.	2	2	Victor	3	3
Marglobe	2	2	Yellow Pear	3	3
Master Marglobe	3	3			

[1] Symptoms recorded on 70 plants of each variety as 1, slight; 2, moderate; and 3, severe.

following varieties showed the severest early symptoms: Bonny Best, Early Rutgers, Master Marglobe, Morse's Special, Early No. 498, Pan America, Supreme Marglobe, Texas Early, Victor, and Yellow Pear. Moderate early symptoms were expressed by Bounty, Chicago, Del Monte, Early Baltimore, Early Prolific, J. T. D., Marglobe, Pritchard Scarlet Topper, Rutgers, and Stokesdale. John Baer, Pritchard, Riverside, and the currant tomato exhibited slight, somewhat delayed, early-phase symptoms. In the later necrotic to moribund phase all varieties continued to respond and show symptom differences as expressed in the early phase, with the exception of Morse's Special Early No. 498 and Texas Early. These two varieties showed only slight necrosis even after 60 days, although in the early phase wilting was severe. John Baer, Pritchard, Riverside, and the currant tomato exhibited the greatest tolerance; Bonny Best, Early Rutgers, Master Marglobe, Pan America, Supreme Marglobe, and Yellow Pear, were the most susceptible and showed the severest symptoms.

OTHER SOLANACEOUS HOSTS

Needle inoculations with the bacterial ring rot organism produced severe wilt and vascular infection in all common garden eggplant (*Solanum melongena* L.) varieties tested and also in the wild spiny scarlet eggplant (*S. integrifolium* Poir.).

A severe flaccid condition of the upper leaves with irregular marginal and interveinal bleaching and retarded leaf expansion was apparent in the inoculated eggplants in from 10 to 12 days. As the systemic infection progressed a conspicuous stunting with retarded and unilateral development of the invaded leaf tissues developed, causing a curving of the leaves. This condition was soon followed by a permanent leaf wilting, bronzing, shriveling, cessation of terminal growth and premature death of the entire plant (fig. 7, *A*). No external stem symptoms were apparent in the inoculated test plants; however, a distinct, creamy bacterial ooze was evident in the vascular tissues on cross section of the stems of infected plants. Histological examination of infected stem tissues shows that the bacteria progress through the xylem elements.

The following commercial eggplant varieties were found to be very susceptible to the bacterial ring rot organism and to exhibit severe progressive systemic symptoms: Black Beauty, Black Bountiful, Black King, Garden Prolific, New Hampshire Hybrid, New York Improved, and Extra Early Dwarf Purple. The causal bacteria were reisolated from each of the varieties and tested in all cases by inoculations to young Triumph potatoes and to tomato seedlings.

In the scarlet eggplant (*Solanum integrifolium*), early systemic symptoms following needle inoculation were evident in from 15 to 18 days as slight flaccidity of the upper leaves. On subsequent days a retarded and unilateral growth of the systemically infected lower leaves developed, the malformed portion at first being a very much darker green and gradually changing to interveinal bleaching, approaching chlorosis. As the chlorotic condition increased very often a well marked, progressive, irregular dendritic necrosis of the branch and anastomosing leaf veins appeared (fig. 8). The tissues gradually became dry and brittle, the leaves died prematurely, and terminal growth ceased.

In repeated tests no symptoms developed, and the ring rot organism was not recovered from inoculated test plants of the following Solanaceae:

Atropa belladonna L.
Browallia americana L. var. Speciosa Major.
Capsicum annuum L. (pepper var. Ruby King).
Datura metel L.
Datura meteloides DC.
Datura stramonium L. (jimsonweed).
Hyoscyamus niger L. (black henbane).
Lycium halimifolium Mill. (matrimony-vine).
Nicandra physalodes Pe (Lr.)s. (apple-of-Peru).
Nicotiana acuminata Hook.
Nicotiana angustifolia Ruiz and Pav.
Nicotiana bigelovii S.Wats.
Nicotiana chinensis Fisch.
Nicotiana glutinosa L.
Nicotiana longiflora Cav.
Nicotiana multivalvis Lindl.
Nicotiana quadrivalvis Pursh.

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FIGURE 7.—Ring rot on eggplant, photographed 25 days after inoculation, plants grown in the same environment. *A*, Black Beauty variety: *a*, Uninoculated control, *b* inoculated plant, showing extreme stunting, unilateral development of leaves and entire plant, chlorosis, and cessation of terminal growth. *B*, Puerto Rican Beauty variety: *a*, Uninoculated plant; *b*, inoculated plant, with only slight interveinal and marginal bleaching and no wilting of lower leaves.

Nicotiana repanda Willd.
Nicotiana rustica L.
Nicotiana rustica L. var. *brasilia* Schrank.
Nicotiana rustica L. var. *humilis*.
Nicotiana sanderae Sander.
Nicotiana sylvestris Speg. and Comes.
Nicotiana tabacum L. (tobacco var. Connecticut Havana No. 38).
Nierembergia hippomanica Miers var. Purple Robe.
Petunia violacea Lindl. var. Violet Gem.
Physalis aequata Jacq. f.
Physalis heterophylla Nees (clammy groundcherry).
Physalis heterophylla var. *ambigua* (Gray) Rydb.
Physalis lanceolata Michx. (prairie groundcherry).
Physalis longifolia Nutt.
Physalis virginiana Mill.
Salpiglossis sinuata Ruiz and Pav. var. Chamois.

FIGURE 8.—Ring rot in scarlet eggplant (*Solanum integrifolium*). Distortion, bleaching, and dendritic necrosis on leaf from systemically infected plant.

Schizanthus wisetonensis Hort. var. Giant Blotched.
Solanum carolinense L. (horsenettle).
Solanum dulcamara L. (bittersweet).
Solanum nigrum L. (black nightshade).
Solanum pseudo-capsicum L. (Jerusalem-cherry).
Solanum rostratum Dunal (buffalo bur).
Solanum triflorum Nutt.

VARIETAL RESISTANCE IN EGGPLANT

Two varieties of eggplant recently developed by Roque and Adsuar,[7] at the Agricultural Experiment Station of the University of Puerto Rico at Rio Piedras were secured for testing in a ring rot inoculation series along with the very susceptible American Black Beauty. The new varieties were Puerto Rican Beauty, a dark purple Black

[7] ROQUE, A., and ADSUAR, J. THE DEVELOPMENT OF NEW VARIETIES OF EGGPLANT RESISTANT TO BACTERIAL WILT. Puerto Rico Univ. (Rio Piedras) Agr. Expt. Sta. Ann. Rpt. 1937–38: 44–45. 1939.

Beauty type of fruit of excellent commercial qualities suited for the export market, and an unnamed selection (E–12), a slender pink-fruited type used for local market. They were developed for resistance to the very serious and destructive soil-borne bacterial disease of the potato and other Solanaceae, bacterial wilt or brown rot (*Bacterium solanacearum* E. F. S.).

In repeated inoculation tests with the bacterial ring rot organism, the two Puerto Rican varieties exhibited a very marked resistance to *Corynebacterium sepedonicum* when compared to the commercial American Black Beauty. The very mild infection that resulted in the inoculated Puerto Rican varieties was expressed by a slight, almost obscure, bleaching of small interveinal and marginal leaf areas in leaves near the point of inoculation, with very little flaccidity, loss of firmness, or wilting of these leaves (fig. 7, *B*). Symptoms of progressive systemic invasion were not observed after the slightly infected lower leaves sloughed. Inoculated test plants of both varieties on being held for long periods in the greenhouse at temperatures favorable for infection showed no further signs of systemic invasion. Neither variety showed any signs of infection on being transplanted into freshly inoculated soil or when inoculum as a suspension was introduced into the soil around the transplants. Inoculated plants on being transplanted to the field showed no wilt symptoms and produced fruit of good quality.

It is important to note that the two varieties of eggplant developed for resistance to a very severe and destructive soil-born bacterial wilt (brown rot) disease also carried a very high degree of resistance to infection by another unrelated bacterial disease of the potato. The two organisms are quite different in their salient physiological and morphological characteristics. *Bacterium solanacearum* is Gram-negative and motile by a single polar flagellum whereas *Corynebacterium sepedonicum* is Gram-positive and nonmotile. The significance of resistance in the Solanaceae to the bacterial ring-rot organism brought out in these preliminary studies is clearly evident.

DISCUSSION

The bacterium (*Corynebacterium sepedonicum*) discussed in this paper has been widespread in the important potato and truck-growing sections of Wisconsin and in recent years has been responsible in many instances for losses in the potato crop. Although the disease has been reported as occurring naturally in the field only on the potato, it is obvious from the results of this investigation and that of other workers, that the bacteria are not specific to this host.

In all commercial varieties of tomato inoculated a decided systemic invasion occurred. The organism was well distributed throughout the vascular elements of affected plants and infection resulted in a typical necrotic or moribund stage.

Thus far the ring rot organism has not been found to overwinter in the soil under Wisconsin conditions, but infected potato tubers as well as infected volunteer potato plants have been found under natural conditions in the field. Ring rot-infected volunteer potato plants may well serve as a very important source of infection for young tomato transplants following a planting of infected potatoes. The planting of tomatoes on fields previously cropped to potatoes that

carried even a trace of ring rot the previous season is not considered safe because of the susceptibility of tomatoes to *Corynebacterium sepedonicum*. Every practicable method of sanitation should be considered and practiced not only in tomato culture but with potato production as well.

The ring rot bacteria are carried on and in the seeds of tomato and in this way may play an active part in the persistence and spread of the causal organism.

In the tissues of the tomato plant and fruit the ring rot bacteria are restricted to the vascular spiral and annular vessels of the xylem; the phloem elements are rarely invaded. The parenchymatous tissues adjacent to the xylem are seldom infected although, when invaded, tissue break-down occurs with the formation of small lysigenous cavities. In contrast, in the tomato canker disease (*Corynebacterium michiganense* (E. F. S.) Jensen) the progressive movement of the organism is chiefly in the phloem tissue, and as a result of outward movement the adjacent meristematic tissues are corroded into extensive cavities. In the tomato brown rot disease (*Bacterium solanacearum E. F. S.*) the organism is primarily a parenchyma-vascular parasite, migrating to surrounding tissues from the xylem, causing extensive tissue disintegration and the formation of large lysigenous cavities in both pith and cortex.

SUMMARY

The ring rot bacterium (*Corynebacterium sepedonicum*) may readily cause severe wilting and death of tomato plants. Detailed histological and reisolation studies have shown that the organism invades the plants systemically through the xylem and seldom affects the adjacent phloem and parenchyma in the initial stages of invasion.

The intercellular spaces of younger xylem tissues show only slight enzymatic action in advance of the bacteria; lignification and the formation of sclereids was not observed.

In infected mature fruit the ring rot organism can be traced from the invaded place of fruit attachment through the tracheae in the placental region and locular cavity directly to the funiculus of the seed. In the seed, the bacteria were identified between the integument and endosperm in the micropylar region between embryo and endosperm. Seed transmission has been demonstrated.

All tomato varieties tested were found to be susceptible although some differences in tolerance were observed.

Infection occurred in all tomato transplants when planted in soil recently infested with suspensions of bacteria. No infection resulted on planting tomatoes in inoculated soil that had been held over winter in the field.

All varieties of the common garden eggplant were found to be susceptible; the annual wild weed, the scarlet eggplant (*Solanum integrifolium*), is also susceptible.

Puerto Rican Beauty and selection E–12, two varieties of eggplant resistant to *Bacterium solanacearum*, are also resistant to *Corynebacterium sepedonicum*.

ANALYSIS OF VARIATION IN PANICUM VIRGATUM [1]

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INTRODUCTION

In the Great Plains region, *Panicum virgatum* L., commonly called switchgrass, is used for grazing, for hay, and for soil-conservation purposes. Because of its economic uses and also because of its wide range of naturally occurring types and its characteristic good seeding habit, switchgrass is considered by some agronomists a desirable forage species.

The recent interest in the expansion and improvement of the grasslands of the United States has resulted in the establishment of a number of forage-plant nurseries in different parts of the country. The wide morphological range of the numerous biotypes assembled in these nurseries as potential breeding stocks is immediately recognized. The study here reported is the result of the writer's efforts to find reliable characters for a logical grouping of the biotypes of *Panicum virgatum* that had been assembled in the grass nursery of the University of Arkansas. It should be recognized that the results reported are an analysis of only the isolates [3] assembled and do not purport to be a critical analysis of *Panicum virgatum* as a whole. They do, however, indicate the complexity of the problem of segregation within this species, of which two varieties and the typical species were recognized by Hitchcock (*18*),[4] who relegated to synonymy other segregates described by other systematists.

MATERIAL AND METHODS

PLANT MATERIAL

Seed and clonal stocks of *Panicum virgatum* were collected or otherwise procured from a number of localities, mostly in the Great Plains region of the United States (table 1). Here the species commonly occurs in association with other tall-grass prairie species. In drier localities it frequents the bottom lands adjoining streams and swales. These seed and clonal stocks were mass-planted in duplicate rows in the grass nursery located on the Agricultural Experiment Station

[1] Received for publication March 24, 1943. Most of the work reported in this paper was conducted in the laboratories of the College of Agriculture, University of Arkansas, Fayetteville, Ark. The investigations were completed at Madison, Wis., in cooperation with the Wisconsin Agricultural Experiment Station and the Division of Forage Crops and Diseases, U. S. Department of Agriculture. Research paper No. 754, Journal Series, University of Arkansas, published with the approval of the Directors of the Arkansas and the Wisconsin Agricultural Experiment Stations.

[2] The writer acknowledges the cooperation extended in procuring seed of *Panicum virgatum*, particularly by members of the Soil Conservation Service, U. S. Department of Agriculture. National Youth Administration students aided materially in the measurements of the several organs reported upon. The author also acknowledges the suggestions and criticisms made by Dr. C. H. Wadleigh, of the U. S. Regional Salinity Laboratory, Riverside, Calif., and by Dr. D. C. Smith and Dr. J. H. Torrie, of the University of Wisconsin.

[3] In this paper the term "isolate" is used to denote any particular segregate or accession collected.

[4] Italic numbers in parentheses refer to Literature Cited, p. 352.

farm, Fayetteville, Ark. The soil of the nursery site, a silt loam of the Newtonia series, is of moderate to low productivity. Plants grown on this site, and others to be mentioned subsequently, comprise the material on which this paper is based. In sampling these rows for the morphological comparisons, the culms were taken at random over the entire length of the duplicate rows.

TABLE 1.—*Sources and chromosome numbers of isolates examined*

Arkansas University accession No.	Chromosone number (2*n*)	Source
1432	18	Chippewa Falls, Wis.
1304	36	Mandan, N. Dak.
345, 345B, 345C, 345D, 1370, 1437, 1596	36	Stillwater, Okla.
1599, 1606	36	Perkins, Okla.
1602	36	Vernon, Tex.
1376, 1411, 1414, 1426, 1429, 1434	36	Chippewa Falls, Wis.
1545	54	North central Arizona.
644	54	Rogers, Ark.
1032	54	Johnson, Ark.
1598	54	Fort Smith, Ark.
1321	54	Greeley, Colo.[1]
1422	54	Chippewa Falls, Wis.
1445	54	Locality unknown.
1047	72	Bentonville, Ark.
1198	72	Canfield, Ark.
966	72	Fayetteville, Ark.
1324, 1328, 1331	72	Greeley, Colo.
1608	72	Amelia, Nebr.
25, 343	72	O'Neil, Nebr.
1329, 1330, 1594	72	Holt County, Nebr.
1305	72	Lincoln, Nebr.
345A, 1500,[2] 1568[2]	72	Stillwater, Okla.
1410, 1413, 1420, 1425	72	Chippewa Falls, Wis.
340, 1322, 1323, 1327	90	Greeley, Colo.[1]
1603	90	Liberal, Kans.
339	90	O'Neil, Nebr.
1328	90	Holt County, Nebr.
1597[2]	90	Perkins, Okla.
1380, 1409, 1416, 1421, 1435	90	Chippewa Falls, Wis.
1325[2]	108	Greeley, Colo.

[1] Original source. Seed used produced in Soil Conservation Service nursery, Lincoln, Nebr.
[2] Perhaps number indicated ±1, 2.

FIXATION AND STAINS

Chromosome numbers were determined in root tips taken from greenhouse-grown plants. These were fixed in a modified LaCour's 2 BD fixative and stained with crystal violet. The drawings of the chromosome complements (see fig. 1, *A*–*T*) were made with the aid of a camera lucida at a projected magnification of 2,750 diameters.

MORPHOLOGICAL MEASUREMENTS

Most of the morphological measurements reported are from plants grown in nurseries in 1938. Exceptions are those isolates reported in table 1 as taken from the Chippewa River terrace near the municipal water-pumping station at Chippewa Falls, Wis. In these cases the material was cut at the ground level in August 1938. The lack of the fourth-node data, where leaves are indicated as being present, was due to stems having been cut above this node, which was at the ground level. Each isolate thus collected was dried and wrapped separately. Later it was moistened and measured in the laboratories at the University of Arkansas. The measurement of each of the organs considered was made as follows.

The height of the culm, expressed in centimeters, was measured from the ground level to the tip of the panicle. The diameters of the first and fourth nodes were arbitrarily selected for measurement with vernier calipers. Lower nodes were not selected, because their accompanying leaves are frequently in poor condition or because, in some instances, some races of this species have none. The length of the uppermost internode was measured from the lowermost branch of the inflorescence to the first cauline node.

The length of the inflorescence was measured from the base of the lowermost branch of the panicle to the tip of the inflorescence. Likewise, the length of the lowermost branch was measured from the insertion of the branch on the central axis to the tip of its most distal spikelet. Measurements were made with a metric rule.

The length of the mature and ripened spikelet was measured from the base of the glumes to the tip of the sterile lemma. This length and the length of the caryopsis were determined with a vernier caliper.

The length of the blades of the first and fourth leaves below the inflorescence was measured from the ligule to the leaf tip; the width was determined 1 cm. above the ligule in order to overcome the natural curvature near the blade base.

The diameters of the rhizomes were determined from measurements made at the nodes; the length of the internode was measured from one scale scar to the next. The diploid and hexaploid isolates and one octoploid isolate had basal caulicles instead of rhizomes and therefore are not considered in the discussion of rhizomes.

No characters that were subject to variations in the judgment of the observers were considered in this study. The evaluation of such plant characters as color, glabrousness or pilosity, and scabrousness or smoothness are subject to personal opinion and bias and were therefore omitted. There is a wide variation in these qualitative characters in *Panicum virgatum*, but a description of them would add little to the verification of the heterogeneity of this species as expressed by quantitative characters. There was also a wide variation in the pathogenic reactions of the isolates studied. This has been amply shown in a recent paper by Cornelius and Johnston (*9*), wherein isolates assembled at Manhattan, Kans., were considered.

STATISTICAL TREATMENT

For purposes of comparison of the different structures, the standard errors of the mean difference among isolates were calculated and used to derive the corresponding t values, whose significance was ascertained from Snedecor's tables (*26*). In the discussion, only differences having odds of 99 : 1 against their being due to random chance are considered as significant.

EXPERIMENTAL RESULTS

CHROMOSOME NUMBER AND MORPHOLOGY

The widespread occurrence of polyploidy in the Gramineae has been indicated in the studies reported by a number of workers (*1*, *2*, *4*, *5*, *6*, *10*, *11*, *19*, *20*, *24*, *25*, *27*, *28*, *29*, *30*), but it was not until recently that polyploidy was known to occur in *Panicum virgatum*. Church (*7*) reported tetraploid and octoploid races of this species in plants

grown from seed produced in Kansas and Oklahoma. In the same paper he reported the tetraploid number 36 for *P. virgatum* var. *spissum* Linder. Church's observations concerning the midwestern material have been fully substantiated in the present study. In addition to tetraploid and octoploid isolates, however, diploid, hexaploid, decaploid, and 12-ploid segregates have been found. In this section of the present paper, the observations concerning chromosome number and morphology will be considered briefly, and these numbers will be referred to in the comparisons of the morphological and nuclear characters in the next section.

One diploid isolate having 18 somatic chromosomes was studied (table 1). The chromosomes were about 0.5μ by 2.0μ and were usually rodlike to somewhat U-shaped (fig. 1, *A*).

Seventeen of the isolates studied were tetraploids. Most of the chromosomes examined were rodlike or U-shaped. Considerable variation was observed in the diameters of the chromosomes in complements of several of these isolates. The figures examined had chromosomes 0.3μ to 0.5μ in diameter and 0.7μ to 2.5μ in length (fig. 1, *B*, *C*, *D*). In isolate 345, introduced from a bulk sample into the grass nursery, four plants decidedly different in appearance from other "sister" plants appeared. These were isolated and designated as isolates 345A to 345D, respectively. One of these divergent forms (345A) was found to have 72 chromosomes and will, therefore, be considered in connection with other octoploid isolates.

Seven hexaploid isolates were examined, and the variations in chromosome size of the individual complements were found to be similar to those seen in the tetraploid complex. The smaller members of complements of the hexaploid complex were mostly 0.3μ by 1.0μ; other members of the same complements were as large as 0.5μ by 2.5μ. An examination of figure 1, *E*, *F*, and *G*, shows that in the hexaploid complex the chromosomes vary considerably in size.

Twenty octoploid isolates were examined. There was a tendency for the individual chromosomes of the complements to be small in diameter, usually about 0.3μ. In length they ranged generally from 1.0μ to 1.5μ, but in isolates 343 and 966 chromosomes ranging from 1.0μ to 3.0μ long were observed. In some isolates (1330, for example), nearly all members of the complements were from 1.0μ to 1.5μ long (fig. 1, *H–P*).

In the decaploid complex, 13 isolates were studied. The chromosomes were usually 0.3μ or less in diameter, but in some complements individual members were larger. They were usually rodlike, less frequently somewhat curved, and from 0.7μ to 1.3μ in length (fig. 1, *Q*, *R*, *S*).

One isolate contained 108 chromosomes. The chromosomes were usually short, 1.0μ or less in length, and about 0.2μ to 0.3μ in diameter. There was some variation in chromosome size in this isolate, as there had been in most of the other number groups (fig. 1, *T*).

The chromosomes of the isolates examined were highly variable in length and diameter throughout the entire series. Those of isolates having higher chromosome numbers tended, however, to be somewhat smaller in diameter than those of isolates having lower chromosome numbers. Reference to the sources of the material from which these isolates were obtained gives no indication of regional segregation of

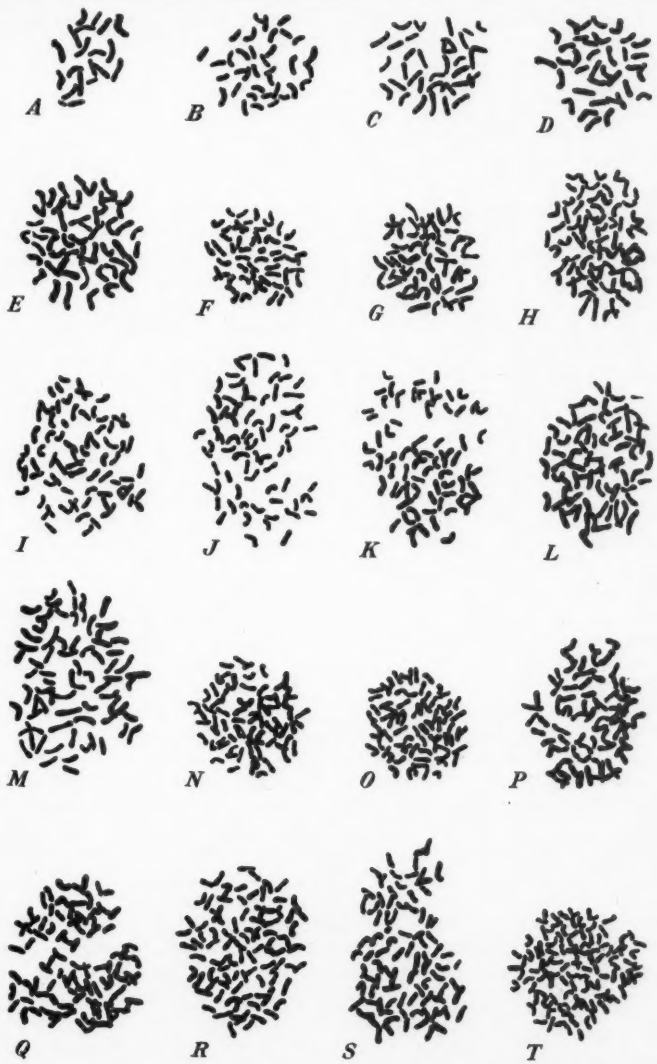

FIGURE 1.—Camera lucida drawings of chromosome complements in somatic tissues. *A*, No. 1432, $2n=18$, Chippewa Falls, Wis.; *B*, No. 345, $2n=36$, Stillwater, Okla.; *C*, No. 345B, $2n=36$, Stillwater; *D*, No. 345C, $2n=36$, Stillwater; *E*, No. 1445, $2n=54$, locality unknown; *F*, No. 644, $2n=54$, Rogers, Ark.; *G*, No. 1321, $2n=54$, Greeley, Colo.; *H*, No. 1198, $2n=72$, Canfield, Ark.; *I*, No. 1331, $2n=72$, Greeley; *J*, No. 1330, $2n=72$, Holt County, Nebr.; *K*, No. 345A, $2n=72$, Stillwater; *L*, No. 25, $2n=72$, O'Neil, Nebr.; *M*, No. 966, $2n=72$, Fayetteville, Ark.; *N*, No. 1413, $2n=72$, Chippewa Falls; *O*, No. 1324, $2n=72$, Greeley; *P*, No. 1608, $2n=72$, Amelia, Nebr.; *Q*, No. 1416, $2n=90$, Chippewa Falls; *R*, No. 1322, $2n=90$, Greeley; *S*, No. 340, $2n=90$, Greeley; *T*, No. 1325, $2n=108$, Greeley. × about 1,900.

type on the basis of chromosome number. Of especial interest is the wide range of chromosome numbers found in the material taken from near Chippewa Falls, Wis. The writer personally collected all of these isolates, and others not yet examined for chromosome number, from an area of not more than 10 acres on the terrace adjoining the Chippewa River, about midway between Chippewa Falls and the city water-pumping station. It is readily conceivable that the many distinct types found in this situation were introduced there through seed in hay fed to horses during logging operations in the early years of this century. If these types were introduced, it may be stated with reasonable certainty that they have successfully established themselves and can be considered as acclimated to Wisconsin weather and soil conditions. It is also possible that these forms have developed through hybridization. If they have arisen as a result of hybridization, it is probable that many of the progeny are still in a stage of genetic instability and that new forms are constantly appearing.

GROSS MORPHOLOGY

Individual comparisons of the organs studied will not be discussed, nor will the comparative sizes of these organs be given in the text. The latter may be ascertained for isolates of the several complexes in the regressions (figs. 2–8). This discussion will only indicate the trends found in the several organs considered and the morphological diversity of the population examined.

The comparisons are summarized in table 2, which shows the number of comparisons made and the number of those significantly different at the 1-percent level. The total population of 28 isolates is referred to as the "A" population, "A" comparison, etc., and the 7 Wisconsin isolates as the "W" population, "W" comparison, etc. These are considered in the following manner: (1) The isolates within a given chromosome complex are compared with all other isolates of the same chromosome complex; (2) all isolates of a given chromosome complex are compared with those of all the other chromosome complexes. This treatment of the data makes it easier to ascertain the heterogeneity within and between chromosome complexes in two types of populations: (1) The total population, which includes all the isolates grown near Fayetteville, Ark., and the isolates collected near Chippewa Falls, Wis.; and (2) the Wisconsin isolates separately. The former population comprises a group of isolates gathered from different topographic, geographic, and edaphic situations and grown together on a single site; incorporated with these are the Wisconsin isolates, also grown on a single site. The treatment of the Wisconsin material as a separate unit affords the opportunity to consider a group of isolates grown and collected from a single area under similar edaphic, topographic, and geographic conditions.

Table 2 shows the total number of comparisons of each organ and the number and percentage found significantly different for isolates of the same chromosome complex and for isolates of the different chromosome complexes. Although these do not bear directly upon the problem under discussion, they serve to illustrate the dispersion of the population in relation to the different characters.

The polymorphism of the population of *Panicum virgatum* studied is readily seen in table 2. For more direct comparison, table 3 gives

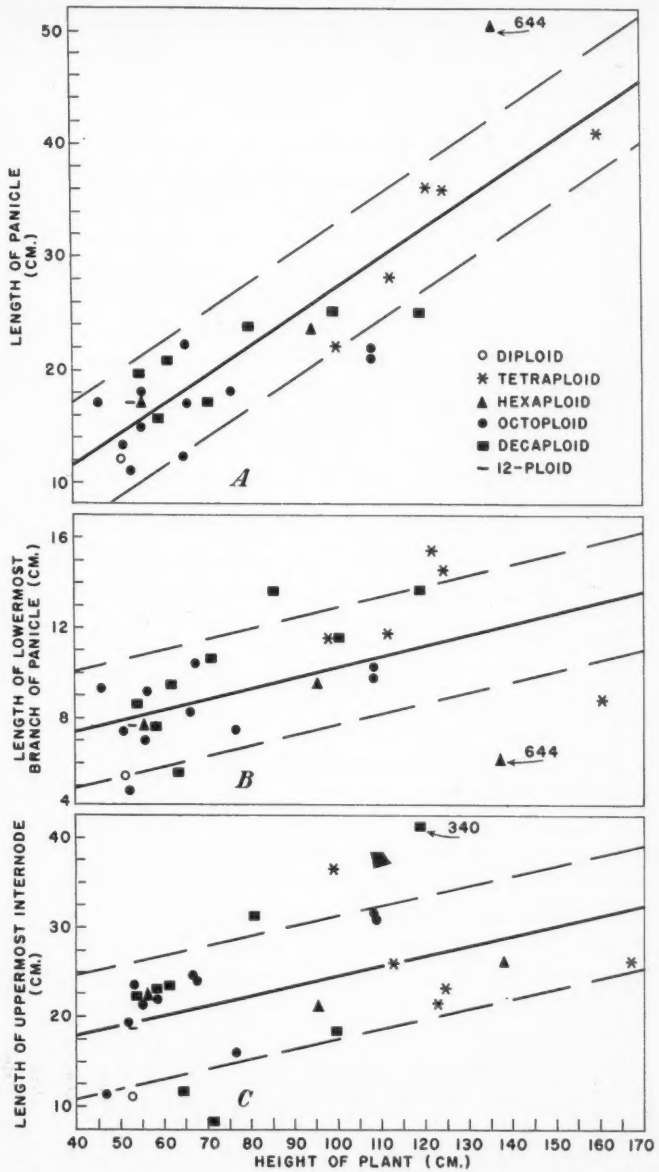

FIGURE 2.—Calculated regression and standard error of estimate for various characters to height of plant. Actual magnitudes of these characters are plotted under the symbols shown. *A*, Length of panicle; *B*, length of lowermost branch of panicle; *C*, length of uppermost internode.

TABLE 2.—*Number of possible comparisons and number and percentage of comparisons significantly different between isolates of the same chromosome number and between isolates of different chromosome numbers in populations A and W of Panicum virgatum*

Comparison	Plant height				Panicle length				Length lower panicle branch				Length first internode				Length first leaf				Width first leaf				Diameter first node				Length fourth leaf			
	A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]		A[1]		W[1]	
2n versus—																																
4n	5	5	1	1	5	5	1	1	5	4	1	1	5	5	1	1	5	5	1	1	5	4	1	0	5	4	1	0	4	4	---	---
6n	3	2	---	---	3	3	---	---	3	1	---	---	3	3	---	---	2	2	---	---	3	2	---	---	3	2	---	---	2	1	---	---
8n	10	4	2	1	10	7	2	2	10	5	2	1	10	9	2	1	10	9	2	2	10	4	2	2	10	5	2	2	10	4	2	2
10n	8	4	3	2	8	7	3	2	8	5	3	2	8	7	3	2	8	7	3	2	8	3	3	1	8	4	3	1	5	4	2	2
12n	1	0	---	---	1	1	---	---	1	0	---	---	1	1	---	---	1	1	---	---	1	0	---	---	1	0	---	---	---	---	---	---
All others	27	15	6	4	27	23	6	5	27	15	6	4	27	25	6	4	26	24	6	5	27	13	6	3	27	15	6	3	21	13	4	4
4n versus—																																
4n	10	7	---	---	10	9	---	---	10	8	---	---	10	5	---	---	10	7	---	---	10	7	---	---	10	7	---	---	6	5	---	---
6n	15	14	---	---	15	13	---	---	15	11	---	---	15	8	---	---	10	6	---	---	15	10	---	---	15	14	---	---	8	6	---	---
8n	50	47	2	2	50	45	2	1	50	32	2	1	50	29	2	2	50	35	2	0	50	42	2	1	50	42	2	0	40	36	---	---
10n	40	36	3	3	40	35	3	2	40	23	3	1	40	27	3	3	40	29	3	1	40	32	3	2	40	30	3	0	20	17	---	---
12n	5	5	---	---	5	5	---	---	5	4	---	---	5	4	---	---	5	3	---	---	5	4	---	---	5	4	---	---	---	---	---	---
All others	115	107	6	6	115	103	6	4	115	74	6	3	115	73	6	6	110	78	6	2	115	92	6	3	115	94	6	0	72	63	---	---
6n versus—																																
6n	3	3	---	---	3	3	---	---	3	1	---	---	3	2	---	---	1	0	---	---	3	2	---	---	3	3	---	---	1	1	---	---
8n	30	25	---	---	30	20	---	---	30	8	---	---	30	15	---	---	20	11	---	---	30	22	---	---	30	22	---	---	20	14	---	---
10n	24	20	---	---	24	15	---	---	24	13	---	---	24	17	---	---	16	10	---	---	24	13	---	---	24	16	---	---	10	7	---	---
12n	3	2	---	---	3	2	---	---	3	0	---	---	3	1	---	---	2	1	---	---	3	2	---	---	3	2	---	---	---	---	---	---
All others	75	63	---	---	75	53	---	---	75	33	---	---	75	44	---	---	50	30	---	---	75	56	---	---	75	56	---	---	40	28	---	---
8n versus—																																
8n	45	29	1	1	45	24	1	0	45	4	1	0	45	28	1	1	45	14	1	0	45	22	1	0	45	19	1	0	45	23	1	0
10n	80	58	6	4	80	45	6	4	80	36	6	3	80	56	6	5	80	39	6	2	80	55	6	4	80	36	6	1	50	33	4	0
12n	10	5	---	---	10	4	---	---	10	3	---	---	10	5	---	---	10	1	---	---	10	5	---	---	10	2	---	---	---	---	---	---
All others	180	139	10	9	180	121	10	7	180	84	10	5	180	114	10	8	170	95	10	4	180	128	10	7	180	107	10	3	120	87	6	2
10n versus—																																
10n	28	20	3	1	28	21	3	3	28	19	3	3	28	25	3	3	28	20	3	2	28	21	3	3	28	17	3	1	10	9	1	0
12n	8	6	---	---	8	4	---	---	8	4	---	---	8	5	---	---	8	3	---	---	8	4	---	---	8	4	---	---	---	---	---	---
All others	160	124	12	9	160	106	12	8	160	81	12	6	160	112	12	10	152	88	12	5	160	107	12	7	160	90	12	2	85	61	6	2
12n versus all others	27	18	---	---	27	16	---	---	27	11	---	---	27	16	---	---	26	9	---	---	27	15	---	---	27	12	---	---	---	---	---	---
Total within complexes	86	59	4	2	86	57	4	3	86	32	4	3	86	60	4	4	84	41	4	2	86	52	4	3	86	46	4	1	62	38	2	0
Percentage differences[2] within complexes	68.6		50.0		66.3		75.0		37.2		75.0		69.8		100.0		48.8		50.0		60.5		75.0		53.5		25.0		61.3		0.0	
Total between complexes	584	466	34	28	584	422	34	24	584	298	34	18	584	384	34	28	534	324	34	16	584	411	34	20	584	374	34	8	338	252	16	8
Percentage differences[2] between complexes	79.8		82.4		72.3		70.6		51.0		52.9		65.8		82.4		60.7		47.1		70.4		58.8		64.0		23.5		74.6		50.0	

Comparison	Width fourth leaf				Diameter fourth node				Length rhizome internode				Diameter rhizome node				Spikelet length				Caryopsis length				Nuclear diameter				Total comparisons and total significantly different		Percentage significantly different	
	A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]		W [1]		A [1]	W [1]	A	W
2n versus—																																
4n	4	4			4	4											5	5	1	1	5	5	1	1	5	5	1	1	62	59	10	8	95.2	80.0
6n	3	2			3	2											2	2			2	2			3	2			35	26			74.3	
8n	10	5	2	2	9	3	2	1									10	10	2	2	10	10	2	2	10	8	2	2	129	83	26	22	64.3	84.6
10n	6	4	2	1	6	4	2	2									8	8	3	3	8	8	3	3	8	5	3	2	97	70	36	25	72.2	69.4
12n																	1	1			1	1			1	1			10	6			60.0	
All others	23	15	4	3	22	13	4	3									26	26	6	6	26	26	6	6	27	21	6	5	333	244	72	55	73.3	76.4
4n versus—																																
4n	6	3			6	4											10	6			10	5			10	5			118	78			66.1	
6n	12	9			12	9											10	6			10	7			15	4			167	117			70.1	
8n	40	36			36	36											50	18	2	1	50	34	2	1	50	24	2	2	616	456	20	11	74.0	55.0
10n	24	19			24	24											40	14	3	1	40	27	3	3	40	25	3	3	468	338	30	19	72.2	63.3
12n																	5	5			5	5			5	5			50	44			88.0	
All others	80	68			76	73											110	48	6	3	110	78	6	5	115	63	6	6	1,363	1,014	60	38	74.4	63.3
6n versus—																																
6n	3	3			3	3			1	1			1	1			1	1			1	1			3	1			33	26			78.8	
8n	30	29			27	20			18	10			18	13			20	14			20	15			30	15			383	253			66.1	
10n	18	14			18	16			16	11			16	9			16	11			16	11			24	15			294	198			67.3	
12n									2	1			2	1			2	2			2	1			3	3			31	18			58.1	
All others	63	49			60	47			36	22			36	23			50	35			50	35			75	39			910	613			67.4	
8n versus—																																
8n	45	27	1	0	36	14	1	0	36	26	1	0	36	21	1	0	45	19	1	1	45	32	1	1	45	29	1	0	648	331	15	4	51.1	26.7
10n	60	45	4	3	54	30	4	2	72	42	6	2	72	47	6	2	80	28	6	1	80	44	6	2	80	55	6	6	1,108	649	84	41	58.6	48.8
12n									9	7			9	6			10	9			10	5			10	8			118	60			50.8	
All others	140	110	6	5	126	89	6	3	99	59	6	2	99	66	6	2	170	79	10	4	170	108	10	5	180	110	10	10	2,354	1,496	130	76	63.6	58.5
10n versus—																																
10n	15	15	1	1	15	11	1	1	28	17	3	2	28	16	3	0	28	8	3	2	28	15	3	1	28	20	3	2	376	254	39	25	67.6	64.1
12n									8	6			8	3			8	7			8	3			8	8			96	57			59.4	
All others	108	82	6	4	102	74	6	4	96	59	6	2	96	59	6	2	152	68	12	5	152	93	12	8	160	108	12	11	2,063	1,312	150	85	63.6	56.7
12n versus all others									19	14			19	10			26	24			26	15			27	25			305	185			60.7	
Total within complexes	69	48	2	1	60	32	2	1	65	44	4	2	65	38	4	0	84	34	4	3	84	53	4	2	86	55	4	2						
Percentage differences [2] within complexes	69.6		50.0		53.3		50.0		67.7		50.0		58.5		0.0		40.5		75.0		63.1		50.0		64.0		50.0							
Total between complexes	414	324	16	12	386	296	16	10	250	154	12	4	250	158	12	4	534	280	34	18	534	355	34	24	584	366	34	32						
Percentage differences [2] between complexes	78.3		75.0		76.7		62.5		61.6		33.3		63.2		33.3		52.4		52.9		66.5		70.6		62.7		94.1							

[1] Number of possible comparisons in first column; number of significantly different comparisons at 1-percent level in second column.
[2] Percentage of statistically significant differences.

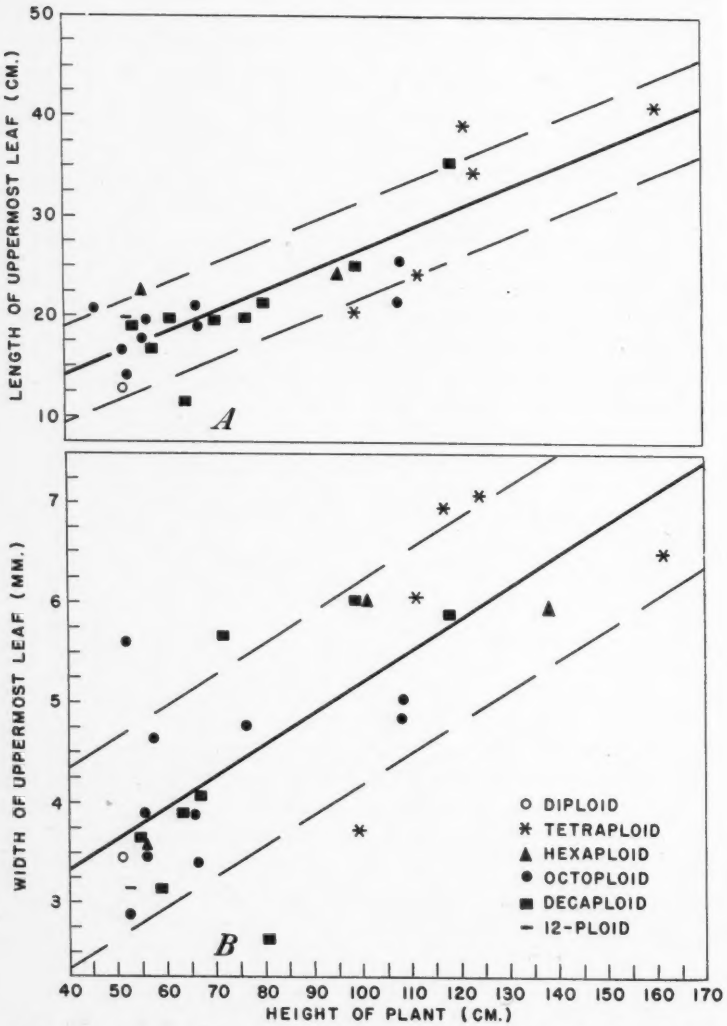

FIGURE 3.—Calculated regression and standard error of estimate for (*A*) length and (*B*) width of uppermost leaf to height of plant. Actual magnitudes of these characters plotted under symbols shown.

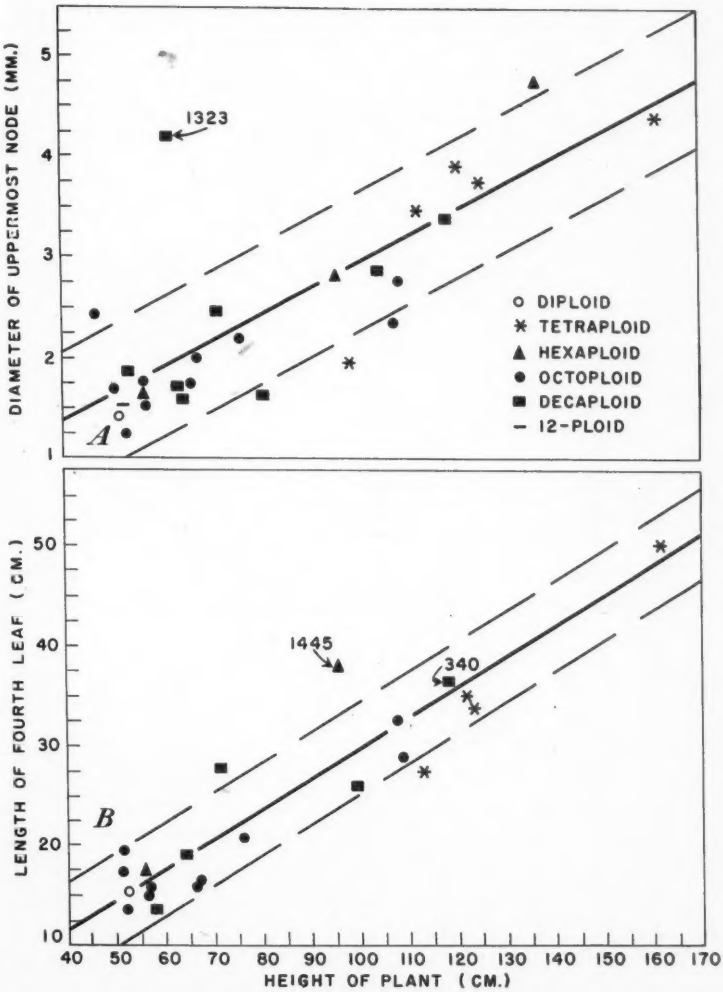

FIGURE 4.—Calculated regression and standard error of estimate for (*A*) diameter of uppermost node and (*B*) length of fourth leaf to height of plant. Actual magnitudes of these characters plotted under symbols shown.

611715—44——3

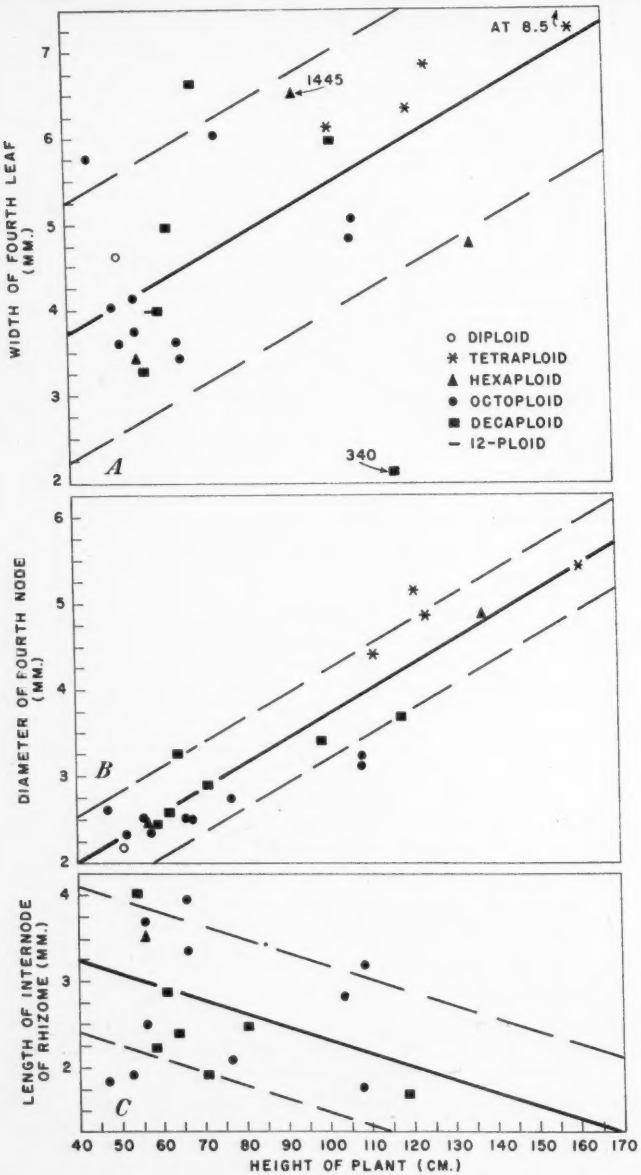

FIGURE 5.—Calculated regression and standard error of estimate for (*A*) width of fourth leaf, (*B*) diameter of fourth node, and (*C*) length of internode of rhizome to height of plant. Actual magnitudes of these characters plotted under symbols shown.

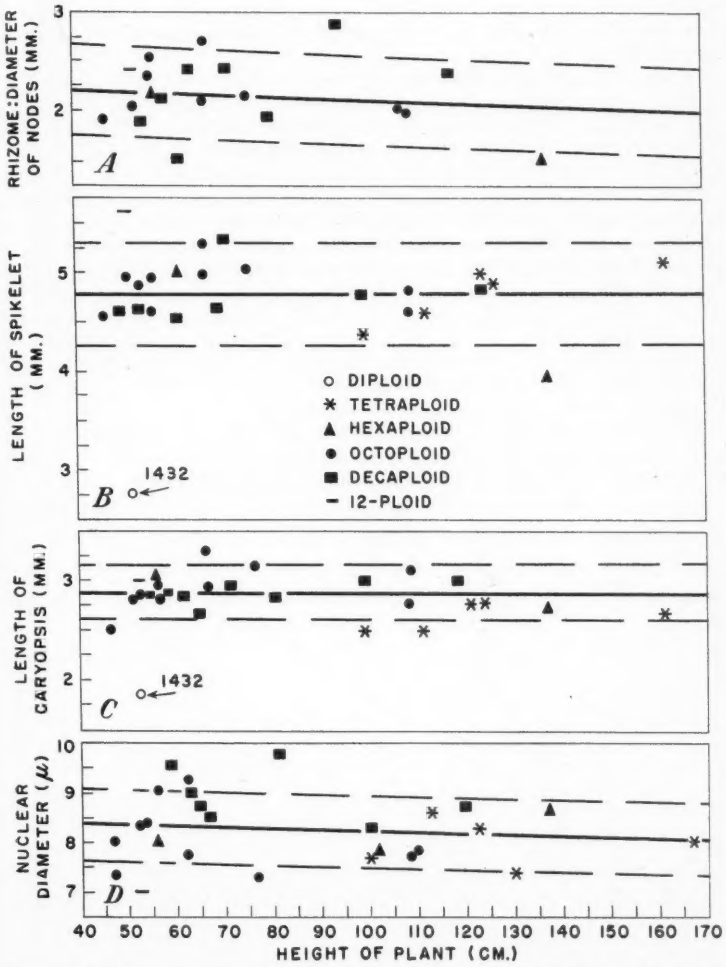

FIGURE 6.—Calculated regression and standard error of estimate for various characters to height of plant. Actual magnitudes of these characters plotted under symbols shown. *A*, Diameter of node of rhizome; *B*, length of spikelet; C, length of caryopsis; *D*, nuclear diameter.

the total number of comparisons made and the percentage of these comparisons that differ at the 1-percent level of significance. The comparison between isolates of different chromosome complexes (table 3) shows that when the "A" isolates are considered for all characters, 66.4 percent of the 7,328 comparisons differed significantly. For the "W" isolates 61.7 percent of the 412 comparisons differed significantly.

Table 3 shows also that 58.6 percent of the total number of "A" comparisons possible between characters of isolates of the same chromosome number differed by a significant amount. Twenty-nine, or 53.7 percent, of the 54 "W" comparisons made were between characters that differed at the 1-percent level of significance.

The spread in the percentage of differences regarded as significant is about 5 percent within complex and between complex pairing. It seems possible that this difference might have been introduced, in part at least, by moving isolates into a locality that differed edaphically, climatically, or topographically from that in which they originally grew. Such a population has in this analysis shown that the comparisons between characters of "A" isolates gave about 5 percent more significant t values than did the comparisons between characters of "W" isolates of the same species, taken from a situation to which those lines are considered naturally adapted.

The data presented for the isolates compared seem to indicate that in a population of plants, of this species at least, more than one-half of the isolates selected differ significantly from one another.

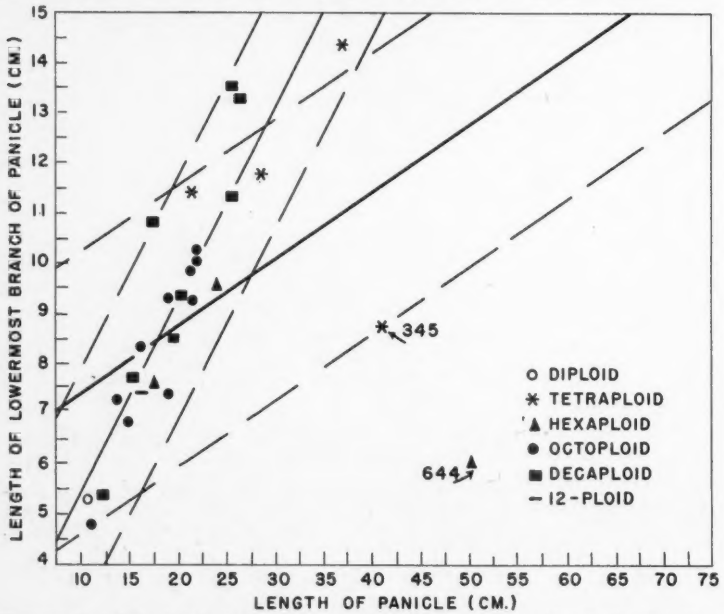

FIGURE 7.—Calculated regression and standard error of estimate for length of lowermost branch of panicle to length of panicle. Actual magnitudes of these characters plotted under symbols shown.

TABLE 3.—*Summary of all comparisons made* [1]

BETWEEN ISOLATES OF DIFFERENT CHROMOSOME NUMBERS

Comparisons	Isolates in population			
	A		W	
	N [2]	Percent [3]	*N* [2]	Percent [3]
Diploids with all others	333	73.3	72	76.4
Tetraploids with all others	1,363	74.4	60	63.3
Hexaploids with all others	910	67.4	0	0
Octoploids with all others	2,354	63.6	130	58.5
Decaploids with all others	2,063	63.6	150	56.7
12-ploids with all others	305	60.7	0	0
Total	7,328	66.4	412	61.7

BETWEEN ISOLATES OF THE SAME CHROMOSOME NUMBER

Tetraploid with tetraploid	118	66.1	0	0
Hexaploid with hexaploid	33	78.8	0	0
Octoploid with octoploid	648	51.1	15	26.7
Decaploid with decaploid	376	67.6	39	64.1
Total	1,175	58.6	54	53.7

[1] 15 characters considered.
[2] *N*=number of comparisons possible.
[3] Percent=percentage of comparisons significantly different at the 1-percent level.

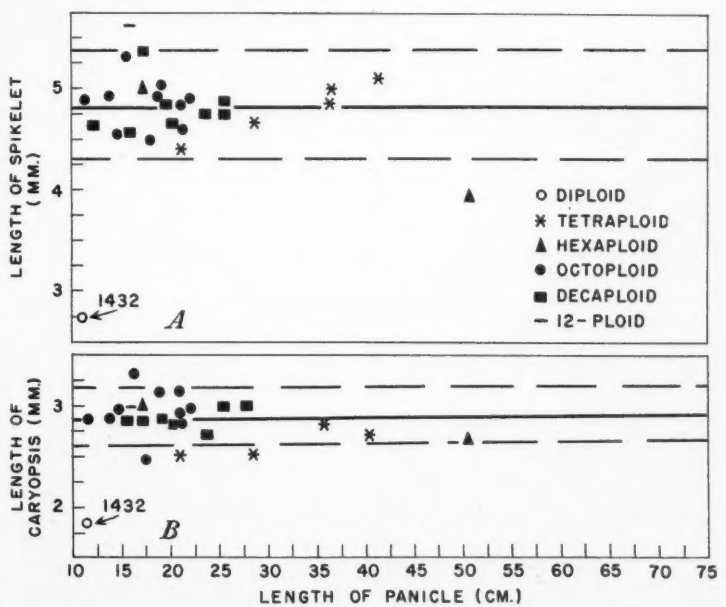

FIGURE 8.—Calculated regression and standard error of estimate for (*A*) length of spikelet and (*B*) caryopsis to length of panicle. Actual magnitudes of these characters plotted under symbols shown.

REGRESSION

In order to ascertain and analyze the variation of several characters with respect to one another, the data were studied by the analysis of variance applied to regression. The regression lines and lines above and below these at a distance of the standard error of estimate are given in figures 2 to 8, inclusive. Of these, figures 2 to 6, inclusive, show the regression of height of plant with the other characters considered. Figures 7 and 8 show the regression of the length of the panicle with the length of the lowermost branch of the panicle, the length of the spikelets, and the length of the caryopsis. The data from which these regressions were plotted are given in table 4, which shows also the *F* values for the regression variance, the regression coefficients, the correlation coefficients, and the coefficients of determination. The means of the characters considered against the height of plant (figs. 2–6) or the length of the panicle (figs. 7 and 8) are plotted on the same charts with the calculated regression lines. In order to ascertain whether there was any clustering of the means within a chromosome number, each isolate of a particular chromosome complex was plotted under a symbol assigned to all isolates of that complex.

TABLE 4.—*Covariance of plant height and of panicle length with measurement of various organs*

COVARIANCE OF PLANT HEIGHT WITH OTHER CHARACTERS

Character	*N*	Variance of errors of estimate	*F* value (variance due to regression)	Regression coefficient	Correlation coefficient	Coefficient of determination
Length of panicle	28	21.40	82.17**	0.256	0.872**	75.96
Length of lowermost branch of panicle	28	5.67	9.15**	.044	.510**	26.03
Length of uppermost internode	28	45.94	7.72**	.115	.479**	22.90
Length of uppermost leaf	27	14.48	69.38**	.206	.857**	73.50
Width of uppermost leaf	28	.83	32.13**	.031	.743**	55.27
Diameter of uppermost node	28	.36	48.45**	.026	.807**	65.08
Length of fourth leaf	22	12.18	149.02**	.291	.939**	88.17
Width of fourth leaf	24	1.65	8.99**	.024	.539**	29.03
Diameter of fourth node	23	.17	109.51**	.028	.916**	83.19
Length of internodes of rhizomes	20	.78	3.50	−.015	−.404	16.29
Diameter of nodes of rhizomes	20	.13	----------	−.001	−.093	.86
Length of spikelet	27	.27	----------	.001	.015	.02
Length of caryopsis	27	.08	----------	.0001	.009	.01
Nuclear diameter	28	.55	----------	−.001	−.062	.38

COVARIANCE OF PANICLE LENGTH WITH OTHER CHARACTERS

Length of lowermost branch of inflorescence	28	6.16	6.38*	0.130	0.444*	19.69
Length of lowermost branch of inflorescence [1]	26	1.25	127.16**	.389	.917**	84.12
Length of spikelet	27	.27	----------	−.001	−.016	.03
Length of caryopsis	27	.08	----------	−.001	−.020	.04

**=odds at least 99:1 against such differences being due to random chance; *=odds at least 19:1 against such differences being due to random chance.

[1] Isolates 345 and 644 removed.

It is noted that if the means of the characters considered are plotted against height of plant or length of panicle or both there is no striking tendency for isolates of a particular chromosome number to occur together. Conversely, the plotted points for the means of characters of isolates of any particular chromosome complex may be scattered along the entire calculated regression for that character.

Theoretically, for any one of the isolates of the population examined to be significantly different from other isolates in regard to a

particular character, the plotted point for that isolate should lie outside at least twice the standard error of estimate from the regression of the whole population. Several isolates differ significantly in this respect and will be considered briefly.

Only one isolate, No. 644, a hexaploid from Rogers, Ark., fell outside of the expected range in the height of plant to length of panicle regression (fig. 2, *A*). This isolate has long panicles, which make up over a third of the height of the plant, and short lowermost branches of the panicle (figs. 2, *B*; 7).

Isolate 340, a decaploid from Greeley, Colo., has long uppermost internodes. The mean length of this character plotted against height of plant lies outside twice the standard error of estimate from the regression (fig. 2, *C*).

Isolate 1323, another decaploid from Greeley, has uppermost nodes that are larger than the expected. Its nodes have a mean diameter of 4.1 mm., whereas the expected diameter of the uppermost culm node for a plant of its height (61.6 cm.) is about 1.8 mm. (fig. 4, *A*).

Isolate 1445, a hexaploid of unknown origin, has fourth leaves somewhat longer than the expected. Their width is, however, within the expected range (figs. 4, *B*; 5, *A*). Contrasting sharply with this relationship is that of isolate 340, which has very narrow fourth leaves (2.1 mm.) whose lengths (36.9 cm.) are near the expected for plants 118.0 cm. high.

The short spikelets and caryopses of the diploid isolate 1432 were both found to lie outside the expected range based upon height of plant (fig. 6, *B* and *C*).

No isolate examined varied from the expected in regard to length or width of uppermost leaves, fourth-node diameter, internode length, rhizome-node diameter, or nuclear diameter (figs. 3; 5, *B* and *C*; 6, *A* and *D*).

Two interesting cases were noted in considering the relation between the length of the lowermost branch of the panicle and the length of the panicle. Isolate 644, already mentioned as having very long panicles relative to height of plant, and isolate 345, a tetraploid from Stillwater, Okla., have very short lowermost panicle branches. If these two isolates are considered as a part of the total population, the calculated regression does not approach the course of the plotted means for the relation of length of panicle to length of lowermost branch of panicle (fig. 7). If, however, these two cases are not included in the total population used for the calculation of the regression, the expected or calculated regression follows the plotted relationship rather closely.

A study of the relation between the length of the panicle and the length of the spikelets and caryopses shows that both of the last-named characters of isolate 1432 were shorter than the expected (fig. 8).

It has been shown by these regressions that (1) there are positive and highly significant regressions between the height of the plant and the magnitude (width, length, or diameter) of the other aerial vegetative characters, (2) the rhizomes are so variable that it is difficult to make any generalization concerning the diameter of their nodes or the length of their internodes, and (3) no relation was found between the height of plant and the length of either spikelets or caryopses.

The relations of the length of panicle to the length of the lowermost branch of the panicle, the length of spikelets, and the length of the caryopses are similar to those between height of plant and aerial vegetative characters or reproductive organs.

These data are in agreement with long-recognized relationships in grasses. Generally one may expect to find that the size of aerial vegetative organs increases in a concomitant manner. Certain exceptions exist, such as those indicated in the preceding data. That there was no relation between such morphological characters as height of plant and length of spikelets and of caryopses was also shown.

NORMAL DISTRIBUTION

In the preceding sections of this paper the chromosome numbers found in several isolates of *Panicum virgatum*, the morphological variability, and the concomitant variability that occurs between certain characters of these isolates were considered separately. In this section, an integration of the results previously indicated will be made. An outline of the plan follows:

(1) The variation between the smallest and the largest means of each of the characters of the isolates examined was considered as 100 percent. The smallest mean value for any particular character in the material examined was considered as at the zero point. The greatest mean value for this particular character would then be at the 100-percent point, and isolates having mean values intermediate between these two would consequently fall along the base line between the zero and the 100-percent point. By this procedure it is possible for a portion of a normal frequency distribution to lie beyond the zero and 100-percent points.

(2) Four isolates, Nos. 345, 1321, 1413, and 340, from the tetraploid, hexaploid, octoploid, and decaploid complexes, respectively, were selected from the total population. The diploid and 12-ploid isolates were not included because of small populations. The following factors were considered in the selection of the isolates: (*a*) The population used to establish the mean height must be sufficiently large to be reasonably accurate for that particular isolate; (*b*) the heights should be reasonably well distributed along the base line for this particular character. For other characters it is then possible to ascertain lateral movement along the base line with relative ease by referring back to normal frequency distribution curves of plant height.

(3) Normal frequency distribution for the magnitude of each of the 15 characters considered was calculated from the frequency distribution of the sample and plotted on the base line in their positions relative to the zero point. To determine these distribution curves, the standard deviations of the means were used; that is, if another sample was taken from the population of a particular isolate and the observations represented by a normal frequency curve, the chances would be 2 to 1 that the mean of that sample would fall within the standard deviation of the curve representing that particular line as presented in this paper. These curves are based upon the standard deviation of the mean rather than upon the standard deviation of the individual because the t values were based upon the standard error of the mean. This type of presentation permits the magnitude of the t value to be shown on the charts by the distance between a given pair of curves for two different races. The following formula was used (*21, pp. 123–150*) for the determination of the normal frequency distribution curves:

$$Y = \frac{N}{\sigma_n \sqrt{2\pi}} \cdot e^{\frac{-x^2}{(\sigma_n)^2}}$$

Here Y is the distance along the ordinate axis beyond the mean; x is the distance along the abscissa beyond the mean; N is the total number of variates; σ_n is the standard error of the mean; e is log. 10^e (0.4343). The points thus derived were plotted to scale, making it possible not only to ascertain and show graphically the relative significance between two curves but also to give a concept of the relative dispersion of the populations considered.

It was assumed that if there was a regular and definite relation between the characters considered one could expect the normal distribution curve of a certain isolate to retain essentially the same relative position between the extremes (0 percent and 100 percent) of the several characters considered. There is no positive evidence that this is true. Inspection of figure 9, *A*, shows, for example, that isolate 1413 was the shortest isolate considered. In length of panicle it is 16 percent of the range above the zero point, or somewhat longer than the isolate with shortest panicles (fig. 9, *B*). The normal distribution of this character of isolate 1321 lies entirely within the limits of the distribution of isolate 1413. A consideration of the length of the lower-

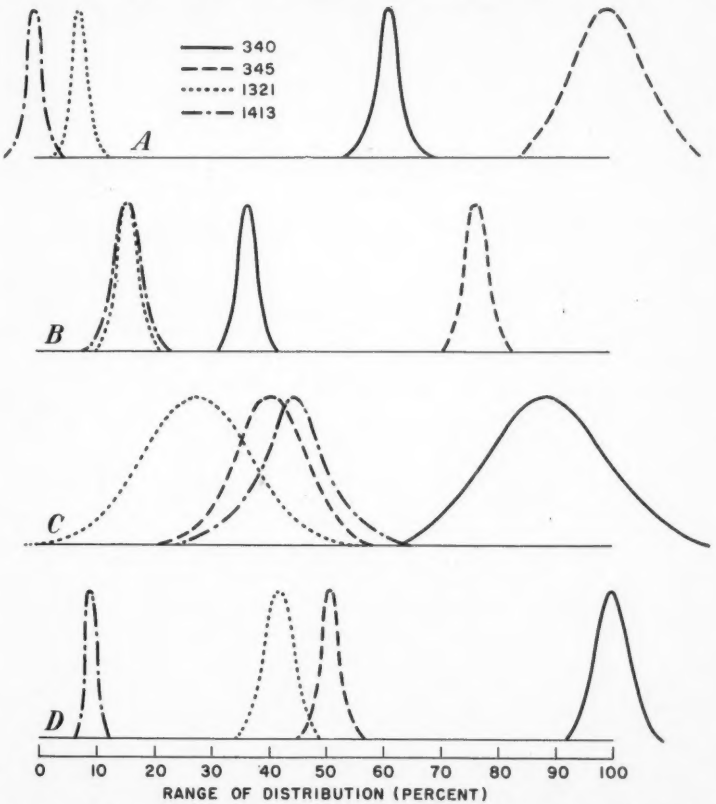

FIGURE 9.—Calculated normal frequency distribution curves for various characters of a tetraploid (345), a hexaploid (1321), an octoploid (1413), and a decaploid (340) isolate. The base line represents 100 percent of the mean range found in any particular character. The frequency distribution curves are arranged according to their sizes relative to the zero point, the mean of least magnitude for each character. The actual range in a given character is given with the character legend. *A*, Plant height (range 46.3–162.2 cm.); *B*, length of panicle (range 11.1–50.5 cm.); *C*, length of lowermost panicle branch (range 4.9–14.5 cm.); *D*, length of uppermost internode (range 5.7–39.4 cm.).

most branch of the panicle (fig. 9, *C*) shows that isolate 1413 has shifted positions in relation to the zero point and, for this character, lies somewhat above isolates 1321 and 345. That other isolates also vary in relation to those with which they are compared may be seen by following isolate 345 through the graphs (figs. 9–12).

The shape of the normal distribution curves indicates the relative dispersion of the characters of the isolates compared (figs. 9–12). As an example of this, one may consider the height-of-plant curves (fig. 9, *A*), which show the relative dispersions about the means for the four isolates. Isolate 345 has a rather broad dispersion as compared with those of other isolates shown. Other striking examples are the dis-

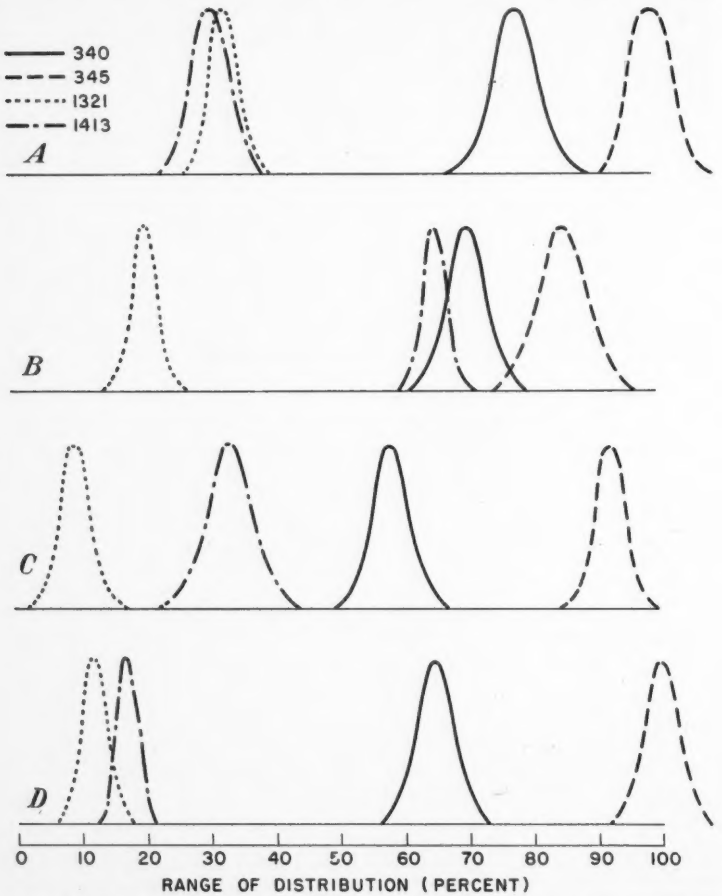

FIGURE 10.—Calculated normal frequency distribution curves for various characters (see fig. 9): *A*, Length of uppermost leaves (range 11.4–40.7 cm.); *B*, width of uppermost leaves (range 2.6–7.1 mm.); *C*, diameter of uppermost nodes (range 1.3–4.7 mm.); *D*, length of fourth leaves (range 12.8–49.8 cm.).

tribution curves for the reproductive organs (fig. 12, *A* and *B*). In the distribution curves for the length of the spikelets, there is but little difference in the dispersion of the four isolates considered. However, the case presented by the length of the caryopses is different. For this character the normal distribution of isolate 1321 lies entirely within the distribution curve of isolate 340. The configuration of the curves for isolates 340, 345, and 1321 are essentially similar and of relatively the same degree of dispersion. However, the distribution of the population of isolate 1413 is markedly different.

DISCUSSION

The data that have been discussed do not appear to aline themselves definitely with some of the observations reported for other species or genera. Investigations by Hagerup (*15*, *16*) and Tischler

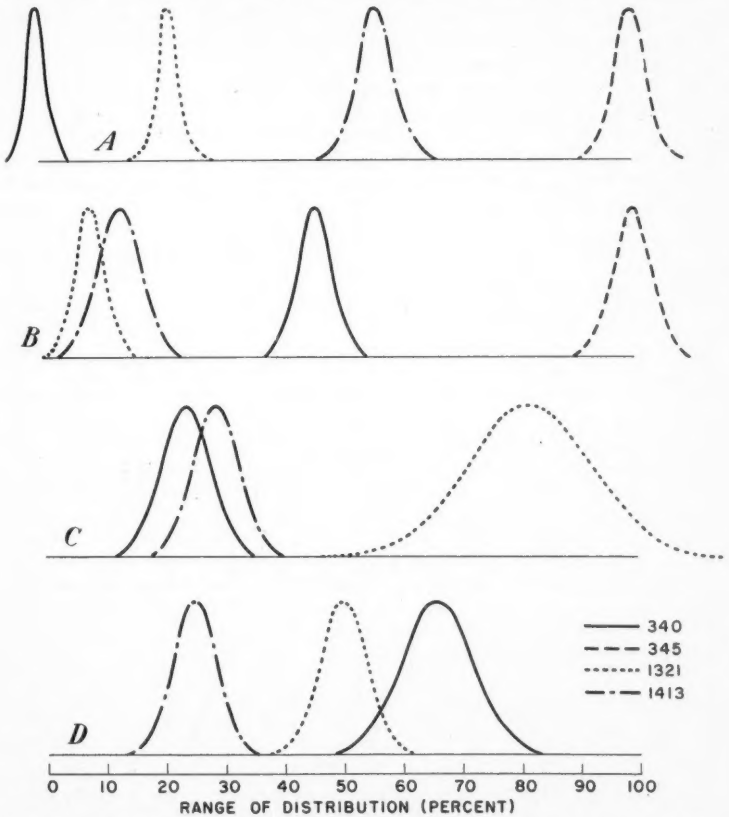

FIGURE 11.—Calculated normal frequency distribution curves for various characters (see fig. 9): *A*, Width of fourth leaves (range 2.1–8.5 mm.); *B*, diameter of fourth nodes (range 2.2–5.4 mm.); *C*, length of internodes of rhizomes (range 0.9–4.1 mm.); *D*, diameter of nodes of rhizomes (range 1.5–2.9 mm.).

(*31*) indicated that the percentage of polyploids is higher in climatically unfavorable regions than in regions where the climate is favorable. Hagerup (*17*) stated that * * * "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid form will not thrive." Müntzing (*22, p. 291*), in discussing autopolyploids, stated:

> Table 1 and the instances given above, however, suffice to demonstrate with absolute certainty that *chromosome races are ecologically different.* Only in one case (No. 18) is the possibility open that the races have the same ecological properties * * *. In fact, *not a single case is known in which it has been demonstrated that intraspecific chromosome races are ecologically identical.* In view of the data reported in table 1, it does not either seem likely that such cases will ever be found.

Certain discrepancies occur between these statements and the observations reported in this paper. The diploid isolate of *Panicum virgatum* was collected from west-central Wisconsin, where the climate must be considered continental. Other tetraploid isolates were found in an area extending from Wisconsin and North Dakota to Oklahoma and Texas. Church (*7*) reported tetraploid isolates of this species from Oklahoma and Massachussetts. Additional evidence that this species does not fall into natural chromosome complex ranges is found in other material collected from near Chippewa Falls, Wis. There material of all except the 12-ploid complex was collected from a small area. Likewise isolates of the hexaploid to 12-ploid numbers, inclusive, came from Greeley, Colo., another region

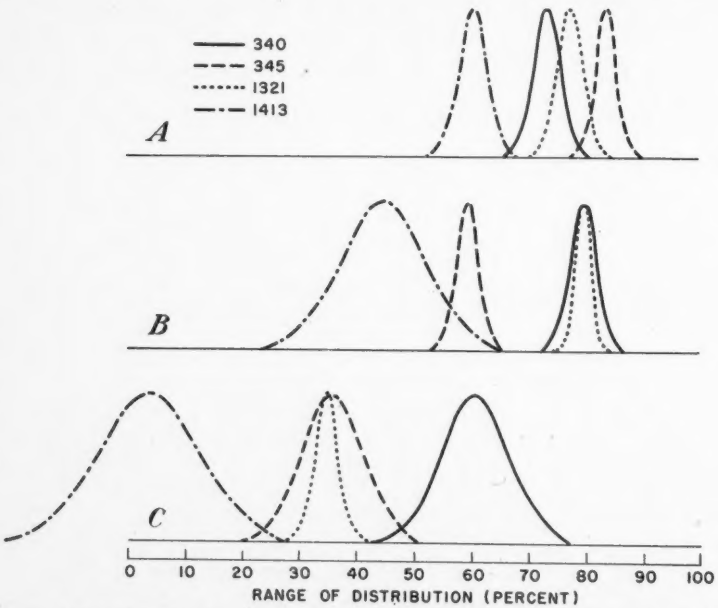

FIGURE 12.—Calculated normal frequency distribution curves for various characters (see fig. 9): *A*, Length of spikelets (range 2.8–5.6 mm.); *B*, length of caryopses (range 1.8–3.3 mm.); *C*, nuclear diameter (range 7.0μ–9.8 μ).

of continental climate. However, these observations support those of Clausen, Keck, and Hiesey (*8*) and Bowden (*3*) in that they show no definite regional segregation of chromosome number. Clausen et al. (*8, p. 423*) stated:

> * * * have emphasized that maritime plants tend to have high chromosome numbers. That this is no general rule is shown by the present investigations, where the coastal species are diploid in three complexes and tetraploid in only one. The investigations on chromosome number and distribution by the Tischler school are based on statistics in which a chromosome number is assigned to each species as a whole, not on counts of races from distinct habitats.

In a subsequent paragraph (*8, p. 424*) it is stated:

> Suggested correlations between chromosome numbers and size, environment, earliness, and other characteristics of plants have proved to be specific, not general.

The large number of different forms within the species *Panicum virgatum* presents a problem as to the taxonomic disposition of the segregates of grass species. There appear to be two methods that could be followed in classifying such segregates, and the success of either can be determined only through actual practice. It is not the purpose of this paper to attempt to outline in detail a definite system that might be workable. It is desirable, however, to suggest and to consider briefly the alternative schemes. The philosophy upon which these arguments are based holds that systematic botany should not be limited to the mere description of segregates but should also include an orderly and thorough analysis of characters and that the results of the investigations should be so presented that they will be of use not only to the specialist but to others as well.

The first of the alternative procedures here suggested as possible would be one whereby the different segregates would be described. This is, and long has been, the procedure generally followed by numerous systematists of both the Old World and the New. From the data presented it will be recognized that upon the basis of a statistical analysis such a procedure would be perfectly valid. It would entail the description of an almost innumerable number of segregates (species, subspecies, varieties, forms, etc.) the rank of which would be dependent to a large extent upon the judgment of the systematist. The importance individual taxonomists place upon different characters would enter as an additional complicating factor. Likewise, it should be recognized that if cytological differences are valid for taxonomic delimitation these differences would have to be, for the most part, ignored. There is little doubt that if such a procedure were followed it would not be long before the taxonomy of the Gramineae would assume the confused condition that now exists in *Crataegus* or *Rubus*. In some genera of grasses this status perhaps has been attained already.

An alternative procedure appears possible which incorporates certain features suggested in recent years by several European workers. This plan would necessitate the recognition of certain species of the Gramineae as being composed of a polyploid system made up of a considerable number of segregates within each one of the chromosome complexes. This concept of a species is essentially similar to Turesson's "coenospecies," but it may or may not be identical with it. Turesson (*32*) proposed the term "coenospecies" and defined it as including "the total sum of possible combinations in a genotype com-

pound." The components of a coenospecies are related, so they may exchange genes among themselves to a limited extent through hybridization (*23*). The delineation of the component races of the polyploid system would not of necessity bear Latinized names but would be generally referred to only under the specific names of the species to which the races belong. The complexes of the polyploid system could be recognized by names such as *diplodium, tetraplodium, hexaplodium*, etc. (*12*). This nomenclature would readily indicate in more critical studies the general nature of the material under consideration. The validity of such a proposal is readily substantiated by reference to previously published papers (*1, 2, 4, 5, 6, 10, 11, 19, 20, 24, 27, 28, 29, 30*). These reports indicate that polyploid systems of chromosome numbers occur within species of *Bromus, Agropyron, Elymus, Paspalum, Andropogon*, and *Spartina*, to mention but a few of the genera wherein the aforementioned condition has been observed. Turesson (*33*) also proposed the term "ecospecies" as "the genotype compound narrowed down to the ecological combination limit." Clausen et al. (*8*) made the following statement in their glossary:

> *Ecospecies*, Turesson's term * * * used to denote a group of plants within the cenospecies whose members are able to interchange their genes without detriment to the offspring. Ecospecies are separated from one another by internal barriers that prevent such free interchange. They may or may not correspond to the Linnaean taxonomic species.

In *Panicum virgatum* this category would include a group of isolates of complexes of the coenospecies specialized to situation such as brackish marshes, open upland prairies, swales, etc. For such segregates of the ecospecies, the terms "ecotype" and "geo-ecotype," depending upon the ecological or geographical distribution, have been proposed (*32*). These isolates are essentially similar to those with which agronomists have been concerned. Gregor (*12*) proposed the term "agrotype" to designate the ultimate crop units that "owe their distinctive characteristics to conscious selection followed by controlled isolation." In the cultivated grasses such as oats, barley, wheat, etc., the broader classes under cultivation would be considered agro-ecotypes (*13, 14*).

Among the more common species of grasses there are commercial varieties or agrotypes, of varying homogeneity, of *Bromus inermis* Leyss. (Superior, Parkland), *Agropyron trachycaulum* (Link) Malte (Mecca, Grazier, Fyra), *Cynodon dactylon* (L.) Pers. (St. Lucie, Common, Giant, Tift), *Phleum pratense* L. (Huron, Shelby, Marietta), *Phalaris arundinacea* L. (Highland, Iowa 503), and *Agrostis palustris* Huds. (Metropolitan, Washington, Seaside, and Coos Bent). These few varieties and others not mentioned are rather distinct morphological types that do not, for the most part, bear Latinized names. The differences that separate them from other races of the same species are perhaps as distinctive as many that have been systematically recognized in species of wild populations. They suggest that frequently the similarity between the varieties of our cultivated species and native grasses has not been fully recognized. The multiplicity of forms of the cultivated grasses introduced from foreign countries in which they are native suggests that analyses of naturally occurring populations of these species would yield results that would essentially parallel those herein reported for *Panicum virgatum*.

SUMMARY

The chromosome numbers of 59 isolates of *Panicum virgatum* were determined. The seed and clonal stocks that were used for this study were taken from an area extending from Wisconsin and Montana south to Arkansas and Arizona. A polyploid series of 18, 36, 54, 72, 90, and 108 somatic chromosomes was determined. There does not appear to be any regional segregation of races of this species upon the basis of chromosome number.

Of the 59 isolates, 28 were compared statistically for the following quantitative characters: Height of the plant, length of the panicle, length of the lowermost branch of the panicle, length of the uppermost internode, length of the uppermost leaf, width of the uppermost leaf, diameter of the uppermost node, length of the fourth leaf, width of the fourth leaf, diameter of the fourth node, length of the internodes of the rhizomes, diameter of the nodes of the rhizomes, length of the spikelets, length of the caryopses, and the nuclear diameters in root-tip cells. In the 7,328 comparisons made between characters of all "A" isolates of different chromosome numbers, 66.4 percent of the comparisons indicated differences exceeding the 1-percent level of significance to occur between those characters. The 1,175 comparisons between all "A" characters of the same chromosome number indicated 58.6 percent of the characters differed to a highly significant degree. One diploid, one tetraploid, two octoploid, and three decaploid isolations obtained from Chippewa Falls, Wis., were considered separately in the same manner as the total population. Of the 412 comparisons made among Wisconsin "W" isolates of different chromosome numbers, 61.7 percent differed significantly. There were 54 comparisons between "W" isolates of the same chromosome number, of which 53.7 percent differed significantly.

The relations between height of plant and all other characters were studied by linear regression. It was ascertained (1) that there was a significant positive regression between the aerial vegetative organs and height of plant and (2) that there was no relation between height of plant and the lengths of spikelets and caryopses, the length of the internodes and diameters of the nodes of the rhizomes, or the diameters of the nuclei of root tips.

Regressions were calculated for the relation of the length of panicle to the length of the lowermost branch of the panicle, the length of the spikelets, and the length of the caryopses. A significant relation was found between the length of the panicle and its lowermost branch. The relations, however, between the length of the panicle and the length of the spikelets and caryopses were not significant.

Normal frequency distribution curves for each of the 15 characters of 4 isolates of different chromosome complexes were calculated and placed upon a common base line representing 100 percent of the variation found in that particular character. These curves indicated that wide differences separate certain lines for some characters, whereas for other characters such lines overlap or may be entirely inseparable.

Alternative procedures that could be followed in the systematic consideration of races of grass species are considered.

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